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EFFECT OF FROST ON WHEAT AT PROGRESSIVE STAGES OF MATURITY

II. COMPOSITION AND BIOCHEMICAL PROPERTIES OF GRAIN AND FLOUR[1]

BY A. G. MCCALLA[2] AND R. NEWTON[3]

Abstract

The increase in dry weight of wheat kernels after flowering accelerated slightly for 14 days, was steady for 10 days, then fell off gradually to zero in about 14 days more, when the kernels weighed 32 gm. per 1,000. At this stage they contained 58 to 60% dry matter, a critical point marking the practically complete cessation of chemical changes.

The weight of nitrogen increased steadily to about 1 gm. per 1,000 kernels at the critical stage. Respiration losses of carbon, before and after harvest, appear to account largely for the changes in the percentage nitrogen in the kernels. The ratio of nitrogenous to non-nitrogenous material moved into the endosperm seems to have been nearly constant throughout the main developmental period.

Ammonia nitrogen first increased to a maximum of 4.8 mg. per 1,000 kernels, then decreased to negligible proportions at the critical stage. Salt-soluble nitrogen in fresh kernels decreased from an initial value of 75% of the total nitrogen to 22% at the critical stage, when a little more than one-third of it was non-protein. Drying the kernels before analysis changed the percentage composition, owing to respiration and synthesis, by an amount varying with rate and conditions of drying.

Frost had no effect on the ash content of the kernels. Four degrees of frost (28° F.) had no effect on the per cent total, salt-soluble or non-protein nitrogen, but 8, 10 and 14 degrees, in cuttings before the critical stage, in both the grain and the flour milled from it reduced the per cent total nitrogen, an effect ascribed to slowing up of respiration, and increased the per cent of the fractions, ascribed to checking of synthesis.

Yields of washed gluten from the control samples were about the same at all stages, but physical properties did not attain maximum quality before the critical stage. Four degrees of frost did not affect yield, but reduced quality in cuttings before the critical stage. More severe frost reduced both yield and quality in immature samples, the effect of the heaviest frost on quality persisting to full maturity.

Both reducing and invert sugar percentages declined in early stages of development. Frost did not affect invert sugar content, but 8, 10 and 14 degrees increased reducing sugars in flours from grain cut before the critical stage. This is ascribed partly to increased enzyme activity, as indicated by greater maltose production, and partly to slowing of respiration by frost injury. Gain in kernel weight by translocation after cutting took place in immature check samples, but not in heavily frozen samples. Respiration losses in the stook were calculated to be about twice as great from the checks as from the heavily frozen samples.

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Introduction

The first paper of this series (18) dealt with the physical characteristics of wheat kernels from plants which had been subjected to graded frost exposures at progressive stages of maturity in the seasons of 1929, 1930 and 1932. In the present paper, the composition and certain biochemical properties of the grain and flour from the 1930 samples are described.

Previous Work

The study of the chemical development of wheat and the effect of frost on this development has received most attention during the last twenty years or so, but there are publications on the subject dating back at least to 1866. The earliest papers to which reference has been found were published by French workers at about this time. Most of the earlier literature dealing with frozen wheat and appearing from 1885 to 1900 is concerned with the feeding value of the frozen grain, but the analyses are of interest. Only a selected group of papers bearing directly on the present investigations will be reviewed.

Fresh and dry weight. The percentage moisture content of wheat kernels has been found to decrease more or less regularly from the time of flowering until maturity, but the actual weight of water has been found to increase at first, soon reaching a roughly constant level, and then to drop rapidly (5, 24, 34). Brenchley and Hall (5) found that the constant-weight period was about 18 days long, while Woodman and Engledow (34) reported it as 10 days. The latter workers further determined that the fresh weight of the kernels increased until rapid desiccation set in, when a distinct change in kernel color from green to yellowish-brown could be noted. The weight of dry matter increased until the same time, but from then on changed little until the final week, during which period a measurable decrease was recorded. The fact that the changes in weight of dry matter are slight after the dry matter reaches 56–60% of the kernel weight has been substantiated by the work of Arny and Sun (1), Olson (20), Saunders (22, 23) and Sharp (24). Arny and Sun, however, report that although changes are slight after this stage has been reached, there is a gradual rise until maturity, but that translocation of materials from the vegetative parts to the kernel ceases as soon as the grain is cut. The latter observation is supported by the work of Wilson and Raleigh (33). Shutt (27) and Saunders (23), in a joint experiment, found that the weight per 1,000 kernels of wheat dried in the stook was higher than that of wheat removed from the heads before drying, in cuttings up to the "firm dough" stage, indicating that there must have been translocation of food materials after cutting. At later stages the relation was slightly reversed.

Total nitrogen. Early workers reported that the percentage total nitrogen in wheat kernels decreased as the grain developed, explaining this by the theory that a larger proportion of the nitrogenous materials is deposited in

early stages, the later filling consisting mainly of starch. Dehérain and Dupont (8) stated that the nitrogenous matter is almost completely elaborated by the time maturation commences, while the starch accumulates in the grain during the last weeks. To explain how starch accumulation could be possible when the only part of the plant remaining green was the upper part of the stem, they demonstrated by laboratory experiments that this part was still capable of actively assimilating carbon from the atmosphere, while by cutting the heads from certain plants at this stage in the field and analyzing the top 15 cm. of the stems 24 hr. later, in comparison with stems of plants left intact, they demonstrated a large accumulation of starch in the stems without heads, with no change in the intact stems. In neither kind of stem was there any change in sugar or nitrogen content. They concluded, therefore, that nitrogen is not being elaborated or translocated at that stage but that the deposition of starch in the kernels is proceeding rapidly. The experimental results obtained by Kedzie (13), and more recently by Arny and Sun (1) and Olson (20) also indicate that there is a fall in percentage total nitrogen during the developmental period.

Most of the modern work is in disagreement with these results, revealing instead that although there is an initial decrease in percentage total nitrogen, this decrease does not long persist, and that a distinct rise occurs toward the end of the developmental period. The amounts of the early decrease and later increase have been found to vary considerably in separate experiments, but the fairly consistent reports of this relation seem to justify the belief in its existence.

Brenchley and Hall (5) described such a course of events, explaining that the pericarp tissues, which are formed first, are rich in nitrogen and that the percentage of nitrogen in the kernels consequently decreases as the less nitrogeneous endosperm is moved in, though the composition of the latter appeared constant during the main filling period. They ascribed the final rise in percentage nitrogen to losses of carbon by respiration. Thatcher (29, 30) accepted this general view, but doubted that respiration could account entirely for the rise in the percentage nitrogen he found in the last stages of kernel development. Woodman and Engledow (34) found the same sequence with kernels analyzed in the fresh condition, as did Saunders (22, 23) with dried material, though in one of his series the ripe sample did not attain the value of the original.

Beginning with Brenchley and Hall (5), various workers have reported their results not only on a percentage basis, but also on the basis of the actual weight of nitrogen and protein in the developing kernels. Saunders (22) found that the protein content per 1,000 kernels rose from 0.884 to 5.104 gm. during development, the rise being most rapid during the early stages, and that the rate of deposition of other materials followed much the same course. Thatcher (29, 30) found similar changes in protein, but a decreasing ratio of carbohydrates to protein after the milk stage.

The effect of respiration on the total nitrogen content of wheat has been studied by McGinnis and Taylor (17) and Tottingham (31). The former workers measured the evolution of carbon dioxide from wheat kernels collected daily, and calculated that about 10 gm. of carbohydrate material per 100 gm. dry matter was used up by respiration during a 16-day ripening period, or sufficient to cause an increase of 1.46% in the protein content of the wheat, unless compensated by fresh carbohydrate material moved into the kernels. While the respiration of threshed kernels may not reflect accurately what occurs in the field, such results do indicate that the loss of carbohydrates is relatively great, perhaps great enough to explain the actual rise found in protein content of wheat under normal conditions. Tottingham found that carbohydrate losses were largely influenced by temperature. Since the ratio of respiration to synthesis was greater at higher temperatures than at lower, the grain grown at the higher temperatures contained a higher percentage protein than that grown under cooler conditions, notwithstanding that the actual amount of protein synthesized was less at the higher temperatures.

Shutt (26) and Harcourt (11) reported that frozen wheat was higher in total nitrogen than non-frozen and, in the belief that proteins are deposited in the kernels earlier than starch, attributed this to the immaturity of the wheat when frozen. The data given in their reports, however, do not appear adequate to support the conclusion that frozen wheat is actually higher in nitrogen. Sharp (25) found the reverse situation during the early stages of kernel development, but after the kernels reached a dry matter content of about 54% there were no differences. Johnson and Whitcomb (12) conclude from their own work and from a survey of the literature that differences in percentage nitrogen content of frozen and non-frozen wheat are due to factors other than frost. They also found that the total nitrogen content of flour milled from frozen wheat bore a normal relation to that of the wheat. Sharp (25) on the other hand, found some of the flours from his frozen samples abnormally high in total nitrogen. This he ascribed in part to milling difficulties with immature wheat, but not entirely, since some flours were higher in nitrogen than the wheat from which they were milled. When the kernels contained 54% or more dry matter at the time of freezing, however, the relation between the nitrogen content of wheat and flour was normal.

Salt-soluble nitrogen. Woodman and Engledow (34) found the percentage of nitrogen soluble in 1% sodium chloride decreased in the developing kernels until these had reached the stage at which they contained about 55% dry matter, and from this point on remained fairly constant. Sharp (24) found that frozen wheat, especially those samples which were immature when frozen, contained higher percentages of nitrogen soluble in 5% potassium sulphate than did the unfrozen check samples.

Non-protein nitrogen. According to Woodman and Engledow (34) the non-protein nitrogen in wheat follows a very similar course to that of the salt-soluble nitrogen except that the amounts are smaller. There was very

little change during the last few days, and the fact that any of these lower compounds remained was ascribed to trapping of them in the rapidly developing tissues where they had no chance of taking part in synthetic processes. These lower nitrogen compounds are reported by Shutt (26), Blish (3), and Johnson and Whitcomb (12) to be present in larger amounts in frozen than in non-frozen wheat, the largest differences being found in the most immature grain. Johnson and Whitcomb suggest that when synthesis has been stopped by frost the non-utilized compounds persist in the dried kernels, while in non-frozen material at a similar stage of development synthesis proceeds to a much greater extent during the drying process. Shutt (26) reported that when frozen and non-frozen wheat was milled, these differences in non-protein nitrogen were not observed in the flour, but Blish (3) and Sharp (24) found the differences still persisted, especially in the more immature samples.

Gluten. Woodman and Engledow (34) report that the most immature fresh kernels showing distinct gluten formation contained 55% dry matter at the time of cutting. The amount of gluten increased as the grain became riper. Crude gluten determinations have been made on frozen and check samples by Whitcomb and Sharp (32) and Johnson and Whitcomb (12). In immature samples they found the dry gluten percentage higher in the checks than in the frozen material, but in all wheat which had reached a dry matter content of 54–56% before it was frozen the value was unchanged by the frost. Whitcomb and Sharp found that in every sample analyzed the per centage dry gluten was less than the percentage crude protein calculated as total nitrogen $\times$ 5.7. Thatcher (28) and Olson (19) had early shown that the relation varied with the strength of the flour, weak glutens losing more by dispersion in washing, while Dill and Alsberg (9) more recently showed the importance of the electrolyte content of the wash water. The ratio of gluten to crude protein has no absolute basis or meaning, as neither substance is pure protein. Gluten varies in composition with the method of washing, while crude protein not only varies in composition with maturity and other factors in the wheat, but its quantity is calculated from the nitrogen content by an arbitrary factor upon the size of which not all chemists agree. The ratio is nevertheless of some industrial interest, both factors being widely used in evaluating flour strength.

Glutenin and gliadin. Recent work in this laboratory (6) and elsewhere (4) has cast doubt on the chemical entity of glutenin and gliadin as separated from gluten by any of the methods so far proposed. A series of determinations of glutenin in the course of the investigations to be reported in this paper showed such unsatisfactory agreement among the three published methods tried, that the results were discarded and the determination discontinued. Though several of the papers already cited in this review give results for glutenin and gliadin, discussion of them does not seem to be justified at present, because of the doubt as to the validity of the methods employed.

Sugars. Brenchley and Hall (5) report the "sugar (dextrose)" content of their earliest samples of wheat kernels, taken about 10 days after flowering, as about 15% of the dry matter, falling rapidly during the course of three weeks to about 2%, at which figure it remained fairly constant for the last fortnight before cutting. By "dextrose" the authors may mean total sugar calculated as dextrose, since when reducing sugar has been reported separately by various authors the quantity has usually been a relatively small fraction of the total sugar, the latter being more of the order of the figures given above. The actual weight of sugar per 1,000 kernels, calculated by Brenchley and Hall, increased during the first few samplings, before dropping to an approximately constant level. These trends agree with those found later by Thatcher (30) who, however, did not begin sampling early enough to note the initial rise in weight.

Blish (3) states that the percentage of reducing sugars is greater in frozen than in sound wheat, and that it increases with the severity of freezing. The data he presents do not, however, separate the effects of immaturity and frost, but rather let it appear that the former was the major influence. The percentage of sucrose was relatively unaffected by either factor. Johnson and Whitcomb (12) also report the combined effect of immaturity and frost on the content of reducing sugar, which varied from 0.09% in flour from normal wheat to 15.04% in flour from the most immature, frosted wheat.

Diastatic activity. During the first fortnight of their sampling period, which began about 10 days after flowering, Brenchley and Hall (5) found the diastatic power of the fresh grain per 100 gm. dry matter, increased, after which it fell steadily. Recalculating their results to show diastatic power per 1,000 grains, they found it remained about constant after the initial rise. Mangels and Stoa (15) and Malloch (14) found a tendency for the diastatic activity of flour to decrease with advancing maturity of the grain from which it was milled. Their series, however, covered only the later stages of kernel development.

The diastatic activity of frosted wheat appears to have been rather neglected, the only reference which has come to our attention being that of Johnson and Whitcomb (12) who found the content of reducing sugar in flours after autodigestion had increased more in those from immature frozen wheat than in those from normal wheat. It is well known, however, that the gassing power of doughs from frosted wheat flour is higher than that of doughs from sound flour.

Ash. It seems to be generally agreed that the percentage of ash in wheat kernels decreases as the grain develops (5, 12, 13, 25, 29, 30, 34). Brenchley and Hall (5), Thatcher (29, 30) and Woodman and Engledow (34) found the weight of ash per kernel increases during this period, though obviously not as rapidly as the total weight of dry matter. The results obtained by Sharp (25) and Johnson and Whitcomb (12) with frozen wheat indicate that the percentage is not affected by freezing.

Experimental Material

The 1930 samples, of which the production and freezing treatment were described in the first paper of this series (18), were used for the main body of analyses reported in the present paper. These samples had been collected on 27 days during the period August 5 to September 18.

To get additional information on the normal course of ripening in wheat, for comparison with the results found with frosted material, the following subsidiary series of 19 samples was collected from the same plot of Marquis wheat in 1930. On July 21, the day on which the heads on most of the central culms were in full bloom, 5,000 heads in as nearly as possible the same condition were marked with small tags. Sampling was begun on July 26 and continued, generally at intervals of two or three days, until September 17, or about two weeks after the wheat was ripe. Collections were made at 8:30 a.m., the heads being cut from the culms, put into a covered can and taken to the laboratory. Approximately 300 heads were collected at the earlier samplings, this number being gradually decreased to 150 as the weight of the grain increased. One-third of the heads were placed at once in an oven with a rapid circulation of air at 60° C. where they were dried for 12 hr. From the remainder, the kernels were promptly removed, half of them being used for analysis in the fresh state, and the other half spread thinly on plates and allowed to dry at room temperature.

For convenience, these 19 subsidiary samples will be referred to as the "Maturity Series," to distinguish them from the main, or "Frosted Series."

Analytical Methods

Dry matter. The dry matter content of fresh kernels, flour samples, and washed gluten was determined by drying the material in a vacuum oven at 98° C. for 48 hr. In dried grain it was determined by drying a 2-gm. sample of ground material in an air oven at 130° C. for 1 hr. Grinding was usually done successively in a Wiley mill and an Arcade mill, a combination which reduced the material to a state of fineness in which it could also be used satisfactorily for nitrogen extractions, more rapidly than could be done with the Wiley mill alone.

Total nitrogen. All total nitrogen determinations were made by the Kjeldahl method, using mercuric oxide as the oxidizing agent and a two-hour digestion period.

Ammonia nitrogen. A 20-gm. sample of fresh kernels was ground as finely as possible with sand and 20 to 50 cc. distilled water in a mortar, then transferred to a distilling flask with enough distilled water to make the volume up to 250 cc. and the ammonia determined by the aeration method used by Plimmer and Rosedale (21) for ammonia in hydrolysates. This aeration method was also applied to dried wheat, after grinding in a mill, but in such material no ammonia could be demonstrated.

Salt-soluble nitrogen. A 10-gm. sample of fresh kernels was ground with sand and approximately 20 cc. of 5% potassium sulphate in a mortar, then transferred to a 250-cc. centrifuge bottle with sufficient 5% potassium sulphate to make the total volume up to 200 cc. The bottle was shaken mechanically for half an hour, then the contents was centrifuged. A 50-cc. aliquot of the clear, supernatant liquid was pipetted into a Kjeldahl flask and used for nitrogen determination. For dried, ground wheat and flour the procedure was modified by using 8 gm. of dry matter and shaking with 200 cc. of 5% potassium sulphate for 1 hr.

Non-protein nitrogen. A 100-cc. aliquot of the supernatant liquid from the above salt extraction was pipetted, with continual shaking, into a flask containing 100 cc. of 5.0% trichloracetic acid. The flask was allowed to stand for half an hour, then the contents was filtered. A 100-cc. portion of the clear filtrate was used for a Kjeldahl nitrogen determination. A discussion of the use of trichloracetic acid as a precipitating reagent will be found in a recent paper (16).

Washed gluten. Gluten was washed from flour samples using tap water which had a pH of approximately 7.5 and a salt concentration of 0.02%. The weight of flour used and the time and rate of washing were those recommended by Dill and Alsberg (9). The phosphate solution recommended by these workers was compared with the tap water, and although the weight of wet gluten obtained was slightly higher when the phosphate solution was used, there were no appreciable differences in the weights of the dry glutens.

Sugars and diastatic activity. The diastatic activity of flour samples was determined by Malloch's modification of Rumsey's method (14). The blank in this determination gives the quantity of reducing sugars present in the flour. An aliquot of the blank was inverted by the citric acid method of Davis and Daish (7) and used for the determination of invert sugar.

Ash. Ground samples of 2 gm. were ashed in a muffle furnace at 570° C. for 6 hr.

Maturity Series

The results with the maturity series are summarized in five tables and five figures, in which the samples are characterized by the number of days from flowering, given in Table I and in four of the figures, and by the dry matter content of the kernels at the time of cutting, given in all the tables.

Fresh and Dry Weights

The progressive development of the kernels is shown in Table I by the changes in the percentage dry matter and in the actual fresh and dry weights of a thousand kernels and their original moisture content. The two sets of dry weights refer to the kernels removed from the heads immediately and *(a)* oven-dried directly, *(b)* air-dried at room temperature, then ground, resampled and oven-dried. The weights of the fresh kernels and of those

TABLE I

FRESH AND DRY WEIGHT OF WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING

Sample No.	Date of cutting	No. of days from flowering	Dry matter at cutting, %	Weight per 1,000 kernels, gm.			Weight of water per 1,000 fresh kernels, gm.
				Fresh*	Oven-dried directly*	Air-dried then oven-dried	
1	July 26	5	26.8	—	—	2.91	—
2	28	7	26.9	—	—	—	—
3	30	9	26.8	22.98	6.16	5.20	16.82
4	Aug. 1	11	26.0	28.22	7.36	6.90	22.66
5	4	14	32.0	36.63	11.72	10.45	26.18
6	6	16	35.7	41.71	14.89	13.27	28.44
7	8	18	40.2	43.98	17.68	16.24	27.74
8	11	21	44.6	46.70	20.83	19.97	26.73
9	13	23	48.9	49.35	24.15	23.43	25.20
10	15	25	50.2	53.48	26.85	25.67	26.62
11	19	29	53.2	55.68	29.63	29.31	26.05
12	22	32	55.7	56.53	31.47	31.31	25.06
13	25	35	58.4	54.33	31.71	32.00	22.62
14	28	38	59.8	54.23	32.41	31.98	21.82
15	30	40	70.4	48.22	33.94	32.44	14.28
16	Sept. 2	43	74.3	43.40	32.26	32.38	11.14
17	5	46	85.3	39.45	33.64	32.22	5.81
18	10	51	82.4	41.04	33.82	—	7.22
19	17	58	85.6	38.88	33.30	—	5.58

**Weights of Samples 3 to 8 calculated.*

oven-dried directly were, in the case of samples 3 to 8, not actually determined, but calculated from the nitrogen contents shown in Table II. The only assumption made was that the weight of nitrogen would not change during air-drying at room temperature in spite of the loss of dry matter by the respiratory oxidation of carbon.

The first four samples were collected while the kernels were still forming, and were small, thin and watery, with a dry matter content of 26 to 27%. In Samples 5 to 7, representing the dry matter range of 32 to 40%, the kernels gained rapidly in plumpness, but the watery condition and original green color persisted. In Samples 8 to 11, dry matter range 44.6 to 53.2%, the kernels changed from watery through milky to a fairly firm dough. The color changed from green through yellowish to a decided pink. These kernels, on drying slowly, acquired a slightly greenish, amber color. Sample 12, cut at 55.7% dry matter, was made up entirely of pink kernels, plump and firm, drying to a clear amber. This was the first sample to develop a tenacious gluten pad when the fresh kernels were ground with water in a mortar. Samples 13 to 17, dry matter range 58.4 to 85.3%, exhibited at the time of collection a gradual change from firm to very hard and from pink to a bright clear amber; all were clear amber when dried. By ordinary visual observation, the wheat was judged fully ripe for binder harvesting at Sample 16, dry matter 74.3%, and the dry weights of the kernels show them to have been fully developed some days earlier. Samples 18 and 19, which were allowed

to stand uncut for one and two weeks after the wheat was fully ripe, were very hard, but had lost the clear brightness of the amber color which characterized the two immediately preceding.

Fig. 1 shows the percentage dry matter and weights per 1,000 kernels in the successive cuttings up to Sample 17, cut on September 5, 46 days after flowering, the samples being numbered correspondingly on the two kinds of

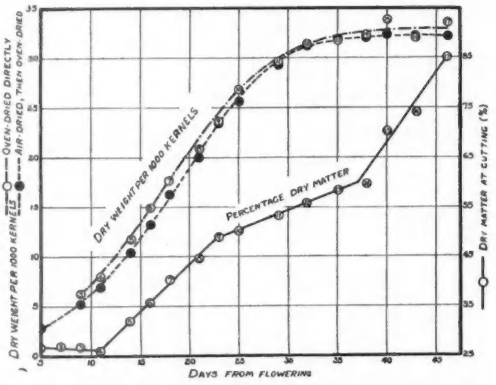

FIG. 1. *Percentage of dry matter in kernels and dry weight per 1,000 kernels at progressive stages of maturity.*

curves. The rate of increase in dry weight accelerated somewhat in the first few samples, then was quite constant up to Sample 10, cut on August 15 at a dry matter content of 50.2%, after which it fell off gradually to maturity. The curve for percentage dry matter is divided into four approximately straight portions. The first four samples came within the period of kernel formation, during which the percentage remained at 26 to 27. The samples from 4 to 9 fall on the second straight portion, marking a period of rapid gain up to 48.9%. From Samples 9 to 14 (59.8% dry matter) the gain is much less rapid, the result of a period of dull, cool, showery weather, aided no doubt by the diminution in the rate of increase in dry weight shown in the other curves. At Sample 14, the percentage curve again begins a steeper ascent, attributable in part to the return of bright, warm weather, but perhaps still more to the onset of rapid desiccation which marks the completion of kernel development. This last part of the curve can probably also be considered straight, the irregularities being more suggestive of sampling errors than of any change in direction. Samples 18 and 19, which were allowed to stand uncut for some time after maturity, are not represented in Fig. 1, but it may be noted (Table I) that Sample 18, which was cut on a damp morning, was 3% lower in dry matter than Samples 17 and 19, which were cut on warm, bright mornings. It may be assumed that with steady weather the percentage curve, after the kernels are fully formed, would approximate more nearly to one straight line than appears in Fig. 1.

The weight of water per 1,000 kernels, shown in the last column of Table I, was fairly constant from the time the kernels were fully formed until they had reached about 56% dry matter, or a period of 18 days, which is the same as that found by Brenchley and Hall (5) at Rothamsted, as compared with 10 days found by Woodman and Engledow (34) at Cambridge. The end of this period marks the attainment of maximum fresh weight by the kernels, and further gains in dry weight were very small. There now ensued a process of rapid desiccation, which is associated with a sharp diminution in the rate, or even the complete cessation, of various physiological processes.

A comparison of the two dry weight curves, which run nearly parallel close together on Fig. 1, affords a certain rough estimate of respiration losses during the curing of grain. The upper curve represents the samples oven-dried directly after removal from the freshly collected heads. The other curve represents those allowed to air-dry in the laboratory before oven-drying. The dry weight of the latter was consistently lower up to Samples 12 and 13. Later irregularities are probably due mainly to sampling errors. The parallelism of the curves below this point suggests that, under laboratory conditions at least, the weight losses due to respiration while air-drying are fairly constant in samples containing less than 56 to 58% dry matter at cutting, though corresponding percentage losses of course become progressively less with increasing weight of the kernels. The constancy in weight losses probably means that the effect of increasing size in the developing embryo was during this period just offset by the diminishing rate of respiration consequent upon the declining percentage moisture content. At later stages the moisture factor became predominant, and the net amount of respiration then declined.

The dry weight per 1,000 kernels, determined by either method, changed very little after the grain reached a dry matter content of 56 to 58%. From this we may conclude that grain cut any time after the kernels have become pink and the dough firm, until full ripeness or even for some considerable time thereafter, will, in the absence of such interfering factors as storms, lodging, etc., give practically the same yield.

Total Nitrogen

The total nitrogen content at progressive stages is shown in Table II as both percentage and weight for the kernels removed from the heads immediately on collection and *(a)* oven-dried directly, *(b)* air-dried at room temperature, then ground, re-sampled and oven-dried; and as percentage only for fresh kernels and for kernels dried in the heads at 60° C. before threshing, grinding and finally oven-drying like the others. In Fig. 2 the percentages found by the three methods of drying, and in the topmost curve of Fig. 3 the weights in the air-dried kernels, are shown graphically.

The percentage total nitrogen in fresh kernels remained about constant while the kernels were forming, then rose gradually but constantly until maturity. In the kernels oven-dried directly, there is perhaps a slight drop in this percentage shown by a comparison of Samples 1 to 3 with Samples 5

TABLE II

TOTAL NITROGEN IN WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING AND METHOD OF DRYING

Sample		In fresh kernels, %	In kernels oven-dried directly		In kernels air-dried then oven-dried		In kernels dried at 60° C. for 12 hr. then oven-dried, %
No.	Dry matter at cutting, %		%	Gm. per 1,000	%	Gm. per 1,000	
1	26.8	0.67	2.51	—	—	—	2.57
2	26.9	0.67	2.50	—	—	—	2.52
3	26.8	0.68	2.54	—	3.01	0.156	2.56
4	26.0	0.73	2.80	—	2.98	0.206	2.58
5	32.0	0.79	2.47	—	2.77	0.289	2.55
6	35.7	0.88	2.46	—	2.76	0.366	2.60
7	40.2	1.00	2.48	—	2.70	0.438	2.54
8	44.6	1.13	2.54	—	2.65	0.529	2.56
9	48.9	1.22	2.50	0.604	2.64	0.618	2.61
10	50.2	1.31	2.61	0.701	2.69	0.690	2.65
11	53.2	1.40	2.63	0.779	2.76	0.809	2.75
12	55.7	1.58	2.83	0.890	2.98	0.933	2.87
13	58.4	1.68	2.88	0.913	3.00	0.960	2.99
14	59.8	1.73	2.89	0.937	3.02	0.966	2.96
15	70.4	2.07	2.94	0.998	2.98	0.967	2.98
16	74.3	2.13	2.87	0.926	2.96	0.958	3.00
17	85.3	2.52	2.95	0.992	3.01	0.969	2.97
18	82.4	2.53	3.07	1.038	—	—	—
19	85.6	2.58	3.02	1.006	—	—	—

to 7 (the value for Sample 4 appears to be in error, and is omitted from Fig. 2). Such a small drop in early stages may be readily assigned to the cause suggested by Brenchley and Hall (5), that the pericarp tissues formed first are richer in nitrogen than the subsequent filling. It is, however, even smaller than the early decrease found by Woodman and Engledow (34) and much smaller than that reported by most workers. From this point onwards there was an almost continuous gain until maturity.

In the kernels air-dried at room temperature before oven-drying, the course of the nitrogen percentages suggests that the large initial decrease found by many workers, and here clearly seen in Fig. 2, is due largely to the method of analysis employed. Samples 3 and 4 (the first two on this particular curve) are as high as the mature samples, and at all stages except maturity the percentage nitrogen is higher than in the samples oven-dried directly. We have seen already (Table I and Fig. 1) that there was a loss of dry weight by respiration during air-drying. The loss of carbon would account for the gain in percentage nitrogen. Since the dry weight losses due to air-drying were fairly constant during the whole period of rapid gain in dry weight (Fig. 1), they would of course increase the percentage nitrogen most in the earliest (lightest) samples, and progressively less as the kernels became heavier. There is reason to believe that if the highest and lowest curve in Fig. 2 could be freed of the influence of experimental error, they would converge with mathematical regularity from kernel formation to maturity. Any

initial drop which may be attributed to changes in the bran-endosperm ratio would affect them both equally, and obviously cannot have played more than a minor part in determining the slope of this part of the curve for air-dried kernels.

In the kernels dried rapidly in the heads at 60° C. the total nitrogen percentages, as may be seen in Fig. 2, occupy an intermediate position between those of the kernels dried by the other two methods. This can be readily

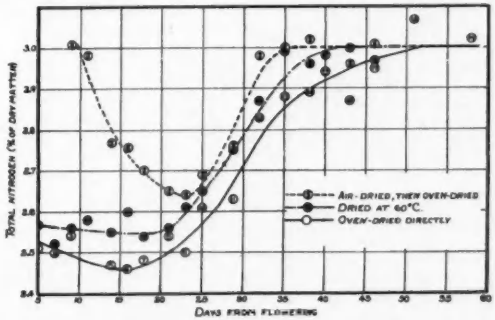

FIG. 2. *Percentage of nitrogen in dry matter of kernels dried in various ways at progressive stages of maturity.*

understood, since respiration losses would be at a minimum under the very rapid drying conditions at 98° C. *in vacuo*, at a maximum under the conditions of slow air-drying at room temperature, and intermediate in a current of air at 60° C. The last-named conditions would give rise initially to intense respiratory activity, but this could not be long sustained because of the rapid loss of moisture.

The material oven-dried directly undoubtedly represents most nearly the actual course of nitrogen percentage in the corresponding wheat standing in the field. The rise which succeeded the initial decline is in agreement with most modern work, and may be attributed largely to respiratory losses of carbon. The magnitude of the rise, over 0.5% nitrogen or about 3% crude protein, is greater than any we have seen reported. However, it will be remembered that McGinnis and Taylor (17) calculated that the loss of carbohydrates during a 16-day ripening period was sufficient to raise the protein 1.46%. That rate sustained over the period of about 30 days represented by the rising portion of the lowest curve in Fig. 2 would account for nearly all of the rise found in these experiments.

The topmost curve in Fig. 2, on the other hand, represents more closely the course of the percentage nitrogen in wheat cut prematurely and allowed to cure in the field. From the practical point of view it appears that whether wheat is cut or allowed to stand uncut after it reaches about 58% dry matter, the total nitrogen percentage attains approximately the same level.

Turning now to the weight of nitrogen per 1,000 kernels (Table II and top curve of Fig. 3), attention is directed chiefly to the figures for air-dried kernels, for which the series of determinations is more complete, and probably more reliable, since respiration should not affect the weight of nitrogen, and the determination was made with dried, well ground and carefully sampled material, as compared with a small quantity of arbitrarily chosen, fresh moist kernels in the case of those oven-dried directly. The agreement between the two methods, where they can be compared in Samples 9 to 17, must be

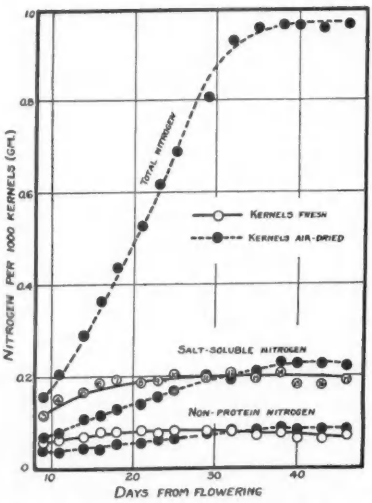

FIG. 3. *Weight of total, salt-soluble, and non-protein nitrogen per 1,000 kernels at progressive stages of maturity.*

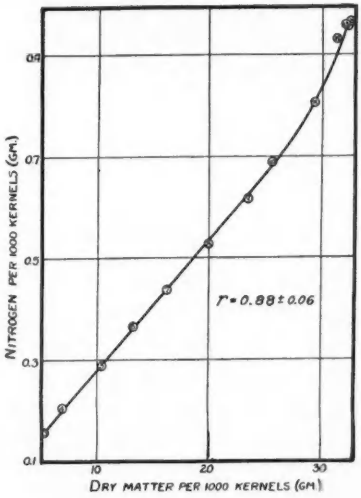

FIG. 4. *Weight of total nitrogen in relation to weight of dry matter in kernels.*

considered fair, considering the difficulties of sampling. The results with the air-dried kernels show a steady increase in weight up to Sample 13, cut 35 days after flowering. There is possibly a slight further gain in Sample 14, cut 3 days later, but it seems safe to say that translocation of nitrogenous matter ceased when the wheat reached a dry matter content of 58 to 60%, a point approximating to that already seen to mark the culmination of various important processes, and for this reason considered critical. The final weight of nitrogen was about 1 gm. in 1,000 kernels.

In Fig. 4, the weights of nitrogen and dry matter in the air-dried kernels are plotted one against the other. From the resulting curve it appears that the ratio of nitrogenous to non-nitrogenous substance in the material moved into the endosperm (assuming a constant fraction of this material was used in the kernel by respiration) was constant during the main period of development. Possibly it was constant during the entire period, as the upward

turn at the end may have been due to a differential change in the rates of gain of total dry substance by translocation and of loss of carbohydrates by respiration, the former diminishing more rapidly than the latter as the moisture content declined.

Ammonia Nitrogen

The analytical results for ammonia nitrogen in the fresh kernels are summarized in Table III, expressed both as percentage of total nitrogen and as weight per 1,000 kernels. From a consideration of the method of analysis and the fact that no measurable amount could be demonstrated in dried kernels, it is obvious that this fraction is free and, from the small and changing amounts present, that it is transitory in nature. The trend observed is a resultant of the relative rates of translocation into, and of synthesis within, the kernels, the latter process being no doubt conditioned largely by the amount of free water present at various stages. Ammonia received by translocation accumulates in the very watery contents of kernels in process of formation. Both percentage and weight increase until the dry matter content of the kernels reaches 32%. The percentage of ammonia nitrogen then begins to decrease —owing either to its utilization or to a disproportionate inflow of other nitrogen fractions—but the actual weight continues to increase up to 40% dry matter, indicating that the rate of utilization has not yet overtaken the rate of translocation. From then on, utilization exceeds translocation, and both percentage and weight of ammonia nitrogen decrease until at 58% dry matter only an insignificant amount is demonstrable. This is the point at which, as we have already shown, translocation of nitrogenous substances to the kernel practically ceases.

TABLE III

AMMONIA NITROGEN IN FRESH WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING

Sample		Ammonia N as % of total N	Ammonia N as weight, per 1,000 kernels mg.
No.	Dry matter at cutting, %		
1	26.8	0.67	—
2	26.9	0.61	—
3	26.8	1.18	1.84
4	26.0	1.28	2.63
5	32.0	1.34	3.87
6	35.7	1.18	4.32
7	40.2	1.09	4.77
8	44.6	0.87	4.60
9	48.9	0.60	3.71
10	50.2	0.56	3.86
11	53.2	0.30	2.43
12	55.7	0.25	2.33
13	58.4	0.04	0.38

It is interesting to note that the quantities of ammonia nitrogen found in these samples, ranging from about 0.4 to 4.8 mg. in 1,000 kernels, were very much less than those reported by Woodman and Engledow (34), and the definite trend seen in our samples does not appear in their results, which show almost as much ammonia in fully mature kernels as at any other stage. No doubt part of the difference at least is attributable to differences in analytical methods.

Salt-soluble Nitrogen

The results of the salt-soluble nitrogen determinations, expressed as percentage of total nitrogen and as weight per 1,000 kernels, are shown in Table IV and Figs. 3 and 5.

TABLE IV

SALT-SOLUBLE NITROGEN IN WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING AND METHOD OF DRYING

Sample		In fresh kernels		In kernels dried at room temp.		In kernels dried at 60° for 12 hr.
No.	Dry matter at cutting, %	As % of total N	As weight per 1,000 kernels, gm.	As % of total N	As weight per 1,000 kernels, gm.	As % of total N
1	26.8	74.1	—	—	—	57.6
2	26.9	72.0	—	—	—	57.9
3	26.8	74.0	0.116	44.5	0.070	51.2
4	26.0	68.6	0.153	39.3	0.081	42.6
5	32.0	58.3	0.169	37.2	0.108	35.3
6	35.7	51.2	0.188	31.2	0.114	31.2
7	40.2	44.3	0.194	29.6	0.130	23.6
8	44.6	35.6	0.187	26.4	0.140	21.9
9	48.9	32.0	0.193	25.4	0.157	19.2
10	50.2	28.7	0.204	24.2	0.167	20.0
11	53.2	25.1	0.196	25.4	0.205	21.1
12	55.7	23.3	0.208	20.8	0.194	18.8
13	58.4	21.5	0.197	22.0	0.211	18.7
14	59.8	22.0	0.207	23.9	0.230	18.9
15	70.4	18.3	0.183	23.5	0.227	22.2
16	74.3	19.7	0.181	23.6	0.226	20.7
17	85.3	19.7	0.195	22.8	0.222	22.6
18	82.4	19.9	0.206	—	—	—
19	85.6	23.2	0.233	—	—	—

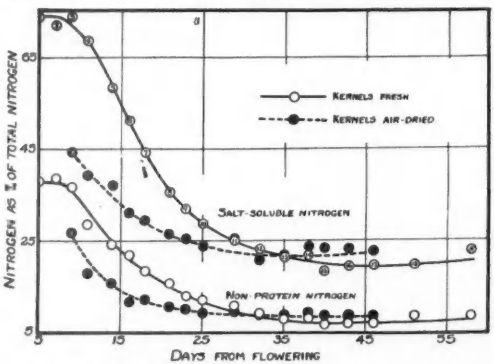

FIG. 5. *Percentages of salt-soluble and non-protein nitrogen in fresh and air-dried kernels at progressive stages of maturity.*

The data for the fresh kernels, which begin with Sample 1, indicate very little change in percentage salt-soluble nitrogen during the early formative stage, but after Sample 3 there was a decided, continuous drop until Sample

13, cut at about 58% dry matter content. Later irregularities in this percentage are probably due mainly to the difficulties of analysis; the grain at this stage was too moist to grind in a mill and yet too hard to grind effectively in a mortar, and it was difficult to ensure complete extraction of soluble nitrogen.

The air-dried kernels showed initially a very much lower percentage of salt-soluble nitrogen than the fresh kernels, owing presumably to the utilization, during the process of drying, of the fraction available for the synthesis of the gluten proteins, and no doubt in part also to a decrease in the solubility of the albumins and globulins on drying. The difference diminished as the grain progressed towards maturity, until at about 58% dry matter (Sample 13) the curves in Fig. 5 intersect. Synthesis and other changes affecting solubility apparently stopped at this point. The fact that the curve for air-dried samples ends on a slightly higher level is no doubt attributable to the difficulty of extracting the fresh kernels already mentioned, which did not obtain in the case of dried kernels. It may be concluded, therefore, that in the mature kernels about 22 to 23% of the nitrogen was salt-soluble.

The grain dried rapidly at 60° C. at first fell into an intermediate position with respect to percentage of salt-soluble nitrogen, then dropped below both the fresh and air-dried kernels. It will be remembered that in percentage of total nitrogen the kernels dried at 60° C. occupied an intermediate position throughout the whole series of samples (Fig. 2). There, however, the comparison was with kernels dried at 98° C. on the one hand and air-dried kernels on the other. Here (Table IV) the comparison is with fresh and air-dried kernels. While it is not clear why the position of the 60° kernels should have changed from intermediate to lowest in early stages, it must be set down as the resultant of processes affected by temperature: synthesis and possibly some denaturation would reduce solubility on the one hand, and hydrolysis increase it on the other. All three classes of kernels represented in Table IV reached approximately the same level in samples cut at 58 to 60% dry matter.

The weight of salt-soluble nitrogen determined in the kernels (Table IV and Fig. 3) increased slowly until about Sample 13, cut at 58% dry matter. Before this, the weight was greater in the fresh than in the air-dried kernels, owing to synthesis or changes in solubility in the latter as already suggested, but afterwards it was slightly lower in the fresh kernels, owing to the difficulty of extraction. The absence of any substantial accumulation of salt-soluble nitrogen in the kernels indicates that it is utilized in the building of higher compounds almost as rapidly as it is produced by synthesis or introduced by translocation. Of about 0.22 gm. salt-soluble nitrogen in 1,000 mature kernels, the figures for non-protein nitrogen given in the next section make it appear that rather less than two-thirds was protein.

Non-protein Nitrogen

The analytical data for non-protein nitrogen are presented in Table V in three ways: as percentage of the total nitrogen, as percentage of the salt-soluble nitrogen (of which fraction it formed a part), and as weight per 1,000 kernels. The first and third of these appear also in Figs. 5 and 3 respectively.

TABLE V

NON-PROTEIN NITROGEN IN WHEAT KERNELS IN RELATION TO MATURITY AT CUTTING AND METHOD OF DRYING

Sample		In fresh kernels			In kernels dried at room temp.			In kernels dried at 60° C. for 12 hr. As % of total N
No.	Dry matter at cutting, %	As % of total N	As % of salt-sol. N	As weight per 1,000 kernels, gm.	As % of total N	As % of salt-sol. N	As weight per 1,000 kernels, gm.	
1	26.8	38.2	51.5	—	—	—	—	50.6
2	26.9	38.8	53.9	—	—	—	—	51.6
3	26.8	36.6	49.5	0.057	26.6	59.8	0.042	46.9
4	26.0	28.6	39.1	0.064	18.1	46.1	0.037	37.2
5	32.0	24.3	41.7	0.070	15.9	42.7	0.046	30.6
6	35.7	22.0	43.0	0.080	11.6	37.2	0.042	22.3
7	40.2	18.5	41.8	0.081	12.2	41.2	0.054	20.5
8	44.6	15.8	44.4	0.083	10.6	40.2	0.056	17.2
9	48.9	13.0	40.6	0.077	10.2	40.2	0.063	13.8
10	50.2	12.1	42.2	0.083	9.3	38.4	0.064	13.6
11	53.2	10.9	43.4	0.083	9.4	37.0	0.076	11.6
12	55.7	9.2	39.5	0.082	9.1	43.8	0.084	10.5
13	58.4	8.0	37.2	0.073	8.7	39.5	0.083	9.7
14	59.8	8.1	36.8	0.078	9.3	38.9	0.090	9.5
15	70.4	6.5	35.5	0.064	8.7	37.0	0.084	8.7
16	74.3	6.9	35.0	0.064	8.8	37.3	0.084	8.0
17	85.3	7.1	36.0	0.071	8.6	37.7	0.084	8.8
18	82.4	8.8	44.2	0.088	—	—	—	—
19	85.6	8.5	36.6	0.086	—	—	—	—

The curves, though necessarily falling lower on the scale than those for salt-soluble nitrogen in the fresh and air-dried kernels, are not unlike the latter and doubtless yield to the same explanations. The 60° kernels (Table V only) are definitely higher than the fresh and air-dried kernels until the samples contained 58 to 60% dry matter at cutting. Again we must suppose the heat to have been the cause of the difference, whether by direct hydrolysis, or by accelerated enzyme activity at 60° C.

Expressed as a percentage of the salt-soluble nitrogen, the non-protein fraction decreased gradually with the development of the kernels, indicating progressively more complete utilization in the synthesis of protein.

In all three classes of kernels, the non-protein nitrogen, which started highest in the 60° kernels, next in the fresh, and lowest in the air-dried, reached by descending gradients varying in steepness the same final level in samples containing over 58 to 60% dry matter, of about 8 to 9% of the total nitrogen, about 37% of the salt-soluble nitrogen, or about 0.08 gm. in 1,000 kernels.

Frosted Series

The 27 cuttings of Marquis wheat at progressive stages of development in 1930, subjected in all but the last case to the freezing treatment described (18), gave a series of 79 samples of grain for analysis. The analytical data reported include the nitrogen fractions and ash content of the grain, and the

nitrogen fractions, washed gluten, sugar content and diastatic activity of the flour. Yield per acre of grain and nitrogen, and some calculations of translocation from straw to grain after cutting, are also reported. In all the tables presented, except Table VI, the data are arranged so that horizontal comparisons show frost effects, and vertical comparisons the effect of progressive development. As usual, the stage of development is indicated by the moisture content of the kernels at the time of cutting. Those samples marked with an asterisk were cut at the north end of the field, where the crop was later and less even in maturity, as a result of soil-blowing in the spring.

Yield per Acre of Grain and Nitrogen

The yield per acre of grain and of its nitrogen content, for all check (unfrozen) samples collected at the south end of the field, where the stand was for the most part quite uniform, are presented in Table VI. The yields from the first ten cuttings were measured directly, but those from the last six cuttings were calculated, because the portion of the block from which they were collected was somewhat uneven and broken. The ratio of the yield per acre in bushels to the weight per 1,000 kernels in grams was about 0.9 in the earliest samples and increased to about 1.1 in Samples 8, 9 and 10. The latter figure was used to calculate the yields from the kernel weights of subsequent samples.

TABLE VI

YIELD OF GRAIN AND NITROGEN PER ACRE FROM CHECK SAMPLES CUT AT SOUTH END OF FIELD

Dry matter at cutting, %	Yield of grain per acre, bu.	Total N in grain, %	Yield of N per acre, lb.
31.2	11.9	3.04	21.7
34.0	12.2	2.98	21.8
34.9	14.7	3.00	26.7
38.1	15.5	3.00	27.9
39.4	19.9	3.11	37.1
40.9	18.9	2.86	32.4
43.9	23.8	2.74	39.1
45.5	26.5	2.76	43.9
46.8	28.1	2.72	45.8
46.7	32.5	2.81	54.8
54.9	36.2	2.87	62.3
56.8	39.2	2.98	70.1
57.3	40.4	2.95	71.5
58.5	40.0	3.02	72.5
65.6	41.0	3.04	74.9
69.4	40.3	3.02	73.0

The kernel weight (yield) reached an approximately constant value by the time the grain contained 57% dry matter, thus agreeing with the result for the maturity series. The yield of nitrogen per acre appears to increase slightly until a later date, but not enough to contradict the conclusion from the maturity series that translocation to the kernels ceases when these reach a dry matter content not greater than 58 to 60%. The practical conclusion from both series is that wheat cut any time between this stage and full ripeness gives about the same yield.

Ash in Grain

The percentage ash in the grain, reported in Table VII, decreased gradually as the kernels developed, the final value being only about half the initial value, in the maturity range studied. Changes in cuttings more mature than

TABLE VII

ASH IN WHEAT GRAIN IN RELATION TO MATURITY AT CUTTING AND EXPOSURE TO FROST

Dry matter at cutting, %	As % of grain (13.5% moist.)			As weight per 1,000 kernels, gm.		
	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	2.56	2.18	2.29	0.346	0.296	0.294
34.9	1.94	1.95	2.11	0.322	0.354	0.358
39.4	1.82	1.88	1.77	0.359	0.386	0.346
43.9	1.58	1.44	1.50	0.369	0.350	0.357
46.8	1.43	1.43	1.51	0.374	0.367	0.391
48.3*	1.45	1.52	1.53	0.346	0.402	0.402
50.5*	1.45	1.40	1.38	0.384	0.405	0.392
51.1*	1.47	—	1.43	0.396	—	0.423
51.3*	1.39	1.39	1.43	0.406	0.390	0.388
55.8*	1.43	1.38	1.37	0.451	0.443	0.448
56.8	1.31	1.35	1.31	0.463	0.485	0.462
57.3	1.33	1.30	1.30	0.501	0.468	0.474
58.1*	1.26	—	1.26	0.442	—	0.447
59.1*	1.29	—	1.37	0.466	—	0.487
65.5*	1.26	—	1.28	0.453	—	0.425
65.6	1.21	—	1.20	0.447	—	0.444
	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	2.08	2.06	2.10	0.302	0.295	0.326
38.1	1.64	1.90	1.94	0.276	0.321	0.359
40.9	1.54	1.76	1.54	0.326	0.373	0.342
45.5	1.54	1.54	1.50	0.364	0.373	0.381
46.7	1.47	1.49	1.41	0.391	0.406	0.380
50.5*	1.38	1.42	1.42	0.383	0.354	0.374
51.1*	1.44	—	1.43	0.387	—	0.423
53.3*	1.40	1.41	—	0.413	0.397	—
54.9	1.31	1.31	1.49	0.451	0.433	0.486
57.4*	1.35	1.33	1.30	0.447	0.451	0.421
58.1*	1.31	—	1.26	0.457	—	0.447
58.5	1.35	1.32	1.38	0.494	0.480	0.497
59.1*	1.35	—	1.37	0.482	—	0.487
65.5*	1.27	—	1.28	0.468	—	0.425
65.6	1.18	—	1.20	0.421	—	0.443
69.4	—	—	1.18	—	—	0.428

**Samples from north end of field.*

are represented by 57–58% dry matter must be regarded as fortuitous, since other constituents became approximately stable in quantity at that stage, and the stooks were protected from rains which might have caused leaching. There are no differences between frozen and check samples which can be attributed to the freezing treatment. These findings are in harmony with hose cited from the literature on an earlier page.

The decreasing ash percentages during the developmental period covered y the data recorded mean of course that during an earlier period minerals were translocated relatively more rapidly than other constituents, and in this later period more slowly. This may be attributed in part at least to the

early laying down of the envelope of the kernel, which is rich in ash. Over a maturity range comparable to that represented in Table VII, it can be seen in Tables I and II that the dry matter and nitrogen content of air-dried kernels both trebled in quantity, whereas the weight of ash (Table VII) increased perhaps 50%.

Nitrogen Fractions in Grain

The total nitrogen, salt-soluble nitrogen, and non-protein nitrogen in the grain, as affected by stage of maturity at cutting and exposure to 4, 8, 10 and 14 degrees of frost, are shown in Table VIII. To save space, these data

TABLE VIII

NITROGEN FRACTIONS IN WHEAT GRAIN IN RELATION TO MATURITY AT CUTTING AND EXPOSURE TO FROST

Dry matter at cutting, %	Total nitrogen, %			Salt-soluble N as % of total N			Non-protein N as % of total N		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	2.94	3.16	3.04	34.7	30.0	30.9	14.2	10.8	10.5
34.9	2.94	3.12	3.00	29.3	27.2	28.7	12.3	10.3	10.3
39.4	2.94	3.00	3.11	28.2	26.0	26.0	12.3	10.3	10.0
43.9	2.68	2.74	2.74	25.4	24.1	25.5	11.6	10.2	10.2
46.8	2.78	2.78	2.72	25.9	25.5	24.3	10.4	10.1	9.2
48.3*	2.60	2.72	2.72	25.0	25.7	25.7	11.5	10.3	9.9
50.5*	2.57	2.58	2.67	24.9	23.3	24.0	11.7	10.5	9.4
51.1*	2.52	—	2.52	24.6	—	25.4	11.1	—	9.5
51.3*	2.55	2.63	2.54	23.5	23.6	25.2	10.6	9.9	9.4
55.8*	2.67	2.68	2.60	25.5	24.6	25.8	9.0	9.0	8.5
56.8	2.84	3.01	2.98	22.5	21.9	21.8	8.5	7.3	7.4
57.3	2.84	2.85	2.95	22.2	21.8	21.7	7.7	7.7	7.5
58.1*	2.76	—	2.76	23.9	—	23.6	9.1	—	8.0
59.1*	2.74	—	2.66	24.1	—	24.8	9.5	—	9.4
65.5*	2.78	—	2.70	23.7	—	25.9	9.4	—	8.9
65.6	3.06	—	3.04	22.9	—	23.4	9.2	—	8.2
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	2.70	2.78	2.98	34.8	35.3	29.5	21.5	18.7	10.4
38.1	2.72	2.70	3.00	30.9	30.4	26.7	18.4	17.0	10.3
40.9	2.66	2.66	2.86	27.4	27.8	25.1	15.0	14.3	9.8
45.5	2.63	2.65	2.76	25.9	25.8	23.9	11.8	11.3	9.1
46.7	2.72	2.71	2.81	26.5	25.1	24.9	11.0	10.7	9.2
50.5*	2.56	2.56	2.65	24.6	23.8	23.4	12.1	10.9	9.8
51.1*	2.47	—	2.52	24.3	—	25.4	11.1	—	9.5
53.3*	2.59	2.58	2.63	25.5	23.3	23.6	12.4	11.6	9.5
54.9	2.80	2.78	2.87	22.1	21.6	21.6	8.6	8.6	7.7
57.4*	2.63	2.65	2.69	24.3	24.1	24.5	9.9	9.3	8.2
58.1*	2.76	—	2.76	23.9	—	23.6	9.1	—	8.0
58.5	3.05	3.00	3.02	21.6	22.0	21.9	7.9	8.0	7.3
59.1*	2.73	—	2.66	24.2	—	24.8	9.5	—	9.4
65.5*	2.78	—	2.70	23.7	—	25.9	9.4	—	8.9
65.6	3.06	—	3.04	22.9	—	23.4	9.2	—	8.2
69.4	—	—	3.02	—	—	—	—	—	—

**Samples from north end of the field.*

are presented only on a percentage basis, since most of the points brought out by the weights per 1,000 kernels have already been discussed adequately in connection with the maturity series. The weights of total nitrogen in some of the samples are, however, used in a later table in calculating translocation and respiration.

The check (unfrozen) samples in this frosted series correspond quite closely to the air-dried samples of the maturity series, the chief differences being that the latter came from heads plucked by hand, rubbed out and air-dried in the laboratory, while the former came from binder-cut swaths, air-dried in the field in stooks protected from weathering. It is observable in Table VIII that in the early cuttings (up to about 48% dry matter) the check samples are slightly higher in per cent total nitrogen than the corresponding air-dried samples in the maturity series (Table II, column 6), and lower than these in salt-soluble nitrogen (Table IV, column 5) and in non-protein nitrogen (Table V, column 6). This means that, as might be expected, drying was slower in the stook than in the laboratory, that consequently the period of physiological activity was prolonged, leading to greater losses of carbon by respiration, thus raising the per cent total nitrogen, and to more complete synthesis, thus reducing the per cent salt-soluble and non-protein nitrogen. In later cuttings the values for corresponding samples in both maturity and frost series are approximately equal. The conclusions from the maturity series are thus fully sustained by the results from the check samples in the frosted series.

Exposures to four degrees of frost had no apparent effect on the per cent total nitrogen or on the salt-soluble and non-protein fractions. Exposures to 8, 10, or 14 degrees of frost, in the earlier cuttings, reduced the per cent total nitrogen and raised the percentages of the fractions. The effect on the total nitrogen can be explained if we assume that frost injury to the germs checks respiration during subsequent drying and thus leads to a higher ratio of carbon to nitrogen in the dried kernels. The same frost injury would check synthesis, and result in the higher percentages observed in the salt-soluble and non-protein fractions. The stage of maturity at which these frost effects ceased to be evident varied with the severity of exposure and was not the same for all quantities measured. Slight differences in non-protein nitrogen appeared to persist to full maturity, but no differences in total or salt-soluble nitrogen, even after the most severe exposures, were found in samples cut with a dry matter content of more than about 57%.

Nitrogen Fractions in Flour

The total nitrogen, salt-soluble, and non-protein nitrogen of the flour milled from the grain samples just described, are given in Table IX, and show almost precisely the same relations to maturity at cutting and frost

exposures. No differences in either grain or flour were found in the wheat exposed to four degrees of frost, while the differences resulting from heavier frost characterized both grain and flour alike.

TABLE IX

NITROGEN FRACTIONS IN FLOUR MILLED FROM FROZEN AND CHECK SAMPLES OF WHEAT

Dry matter at cutting, %	Total nitrogen, %			Salt-soluble N as % of total N			Non-protein N as % of total N		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	2.76	3.08	2.97	21.0	18.8	19.9	8.7	6.2	6.1
34.9	2.74	2.86	2.88	20.1	17.5	18.1	7.7	5.6	6.2
39.4	2.90	2.94	3.10	17.2	14.6	15.5	6.2	4.8	5.2
43.9	2.59	2.58	2.70	18.5	15.5	14.8	6.6	5.0	4.4
46.8	2.68	2.74	2.74	16.4	15.3	15.7	6.0	5.5	5.1
48.3*	2.46	2.60	2.60	17.1	17.3	18.1	6.9	6.5	6.5
50.5*	2.42	2.44	2.58	16.5	15.2	14.7	7.4	5.9	5.4
51.1*	2.34	—	2.48	17.9	—	16.1	7.7	—	6.0
51.3*	2.47	2.41	2.40	17.4	16.6	16.7	7.7	6.6	6.2
55.8*	2.70	2.63	2.68	15.6	15.6	15.2	7.4	6.1	5.2
56.8	2.68	2.82	2.93	13.4	12.8	13.3	5.2	4.3	4.8
57.3	2.63	2.67	2.67	14.4	15.7	14.2	5.0	5.2	4.5
58.1*	2.50	—	2.74	12.8	—	14.9	4.8	—	5.1
59.1*	2.58	—	2.50	14.5	—	15.2	6.4	—	5.6
65.5*	2.52	—	2.52	16.7	—	16.7	5.6	—	5.2
65.6	2.83	—	2.84	15.1	—	14.4	4.6	—	4.2
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	2.56	2.60	2.82	27.3	26.5	18.4	16.4	13.5	6.0
38.1	2.56	2.56	2.84	25.3	22.7	17.3	14.1	12.5	6.3
40.9	2.56	2.50	2.73	19.9	19.6	15.4	10.2	8.4	5.1
45.5	2.52	2.46	2.64	17.5	16.7	15.2	7.5	6.5	5.3
46.7	2.60	2.63	2.74	15.4	14.8	17.5	6.5	6.8	5.5
50.5*	2.45	2.42	2.52	18.8	17.4	17.5	8.6	7.4	6.3
51.1*	2.40	—	2.48	18.3	—	16.1	9.2	—	6.0
53.3*	2.49	2.51	2.46	20.4	18.3	17.9	9.6	8.0	6.5
54.9	2.48	2.50	2.46	17.7	16.4	17.5	6.5	5.6	5.7
57.4*	2.46	2.49	2.46	14.6	12.0	13.8	6.3	5.2	4.9
58.1*	2.50	—	2.74	12.4	—	14.9	5.2	—	5.1
58.5	2.82	2.79	2.82	11.3	11.5	11.7	5.0	5.0	4.6
59.1*	2.58	—	2.50	15.1	—	15.2	6.2	—	5.6
65.5*	2.42	—	2.52	16.9	—	16.7	6.2	—	5.2
65.6	2.83	—	2.84	15.5	—	14.4	5.3	—	4.2
69.4	—	—	2.84	—	—	14.8	—	—	4.2

**Samples from north end of the field.*

The fact that no chemical differences could be detected between lightly frozen and check samples, even though the former suffered external changes and a lowering of grade (18), while heavy frost caused in immature samples chemical changes which persisted in the flour, possibly explains some of the conflicting statements in the literature reviewed earlier in this paper. Investigators would be led to different conclusions, depending upon the stage of maturity and severity of exposure of their experimental material.

Washed Gluten

The results of the washed gluten determinations are given in Table X. The modification of Dill and Alsberg's method already described was used for all flours except those from wheat of the first four cuttings exposed to 10

TABLE X

Washed gluten of flour milled from frozen and check samples of wheat

Dry matter at cutting, %	Dry weight as % of flour			Texture and tenacity			Color			Gluten / Flour protein**		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	16.0	15.8	15.9	1	3	6	1.5	1.5	2	1.02	0.90	0.94
34.9	14.6	17.6	17.2	2	4	6	1.5	2	2.5	0.94	1.08	1.05
39.4	16.0	19.6	17.5	3	4	6.5	2	2	2.5	0.97	1.17	0.99
43.8	14.7	16.1	17.5	2	4	7	2	2.5	3	0.99	1.10	1.14
46.8	16.0	16.4	16.2	3	5	7.5	2	2.5	3	1.05	1.05	1.04
48.3*	14.9	16.8	17.0	3	4	7	1.5	2	2.5	1.06	1.14	1.15
50.5*	14.0	14.1	16.3	4	5	7.5	2.5	3.5	4	1.01	1.01	1.11
51.1*	13.4	—	15.0	5	—	7.5	2.5	—	4	1.01	—	1.06
51.3*	14.2	15.0	14.4	5	7	8	2.5	3.5	4	1.01	1.09	1.05
55.8*	17.2	16.3	17.0	6	7	8	3	4	4.5	1.12	1.09	1.11
56.8	15.1	18.8	18.9	6.5	8.5	9.5	3	4	5	0.99	1.17	1.13
57.3	17.6	19.6	19.6	7	9	10	4	5	5	1.17	1.29	1.29
58.1*	17.6	—	16.2	8	—	9	4.5	—	5	1.24	—	1.04
59.1*	16.7	—	16.0	9	—	9	5	—	5	1.14	—	1.13
65.5*	17.4	—	16.3	10	—	10	5	—	5	1.21	—	1.13
65.6	17.5	—	17.5	10	—	10	5	—	5	1.09	—	1.08
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	10.8	—	17.8	0	—	6	0	—	2	0.74	—	1.16
38.1	13.3	12.0	17.6	0	0	7	0	0	2.5	0.91	0.82	1.09
40.9	16.0	14.4	17.3	0	0	7	0	0	3	1.10	1.01	1.11
45.5	18.1	18.3	15.5	0	0	7.5	0	0	2.5	1.26	1.31	1.03
46.7	15.6	14.6	18.0	1	0.5	7.5	0	0	3	1.05	0.97	1.15
50.5*	15.3	12.9	16.4	1	2	7	1	1	3	1.09	0.93	1.14
51.1*	13.6	—	15.0	2	—	7.5	1	—	4	0.99	—	1.06
53.3*	15.6	14.0	16.8	3	5	8	2	3	4	1.10	0.98	1.20
54.9	16.4	17.9	19.6	5	6	9	2	3	4	1.16	1.26	1.40
57.4*	16.6	15.6	16.6	5	7	8.5	4	4	4.5	1.19	1.10	1.19
58.1*	16.0	—	16.2	7.5	—	9	3.5	—	5	1.13	—	1.04
58.5	17.9	18.2	19.0	8	9	10	4	4.5	5	1.11	1.14	1.18
59.1*	15.0	—	16.0	8	—	9	4	—	5	1.02	—	1.13
65.5*	16.5	—	16.3	9	—	10	5	—	5	1.20	—	1.13
65.6	17.3	—	17.5	9.5	—	10	5	—	5	1.07	—	1.08
69.4	—	—	17.8	—	—	10	—	—	5	—	—	1.10

**Samples from north end of field.*
***Protein = total nitrogen × 5.7.*

and 14 degrees of frost. To avoid complete dispersion of the dough during washing, it was necessary in these cases to use Olson's method (19) of blending the samples with equal parts of a strong flour. While by this method substantial quantities of "gluten" were recovered, it may be doubted that the material was entitled to such a classification, since by itself it possessed none

of the physical characteristics of gluten. At best, the quantities in the first three cuttings were well below those recovered from the corresponding checks without the necessity of blending.

The physical properties of the gluten were difficult to score, because some samples which became slack while being washed yielded a firm tenacious gluten when freed from starch, while others remained slack. The final product from samples which slackened during washing was always spongy in texture, with coarse vesiculation. Texture and tenacity were scored 0 in gluten from flours which could be washed only when blended; 5 when the gluten was firm and tenacious but coarse in texture; 10 when the gluten was firm, tenacious and fine textured; and intermediately for intermediate conditions. Color scores ranged from 0 to 5, 0 being given to the very dark, grey gluten from severely frozen immature samples, and 5 to the creamy gluten from mature material.

The tendency of poor gluten to disperse readily into particles of various sizes was demonstrated by washing the dough of immature, froze nsamples over screens of various sizes, from which the loose material caught could be collected from time to time. For one sample the results were as follows:

Screen	Yield, %	Texture	Color
30 mesh	1.2	1	4.5
64 mesh	6.8	1	2.5
12XX bolting cloth	14.6	0.5	0

The texture of the product did not vary much, but the color of the fraction most resistant to dispersion was very much better than that of the rest. The bolting cloth used as a routine part of the procedure in washing all samples could usually be omitted with little if any loss, but was quite necessary to secure a satisfactory yield of these samples which became very slack during washing.

The yields of dry gluten obtained from the check samples, while they show considerable variation which must be attributed to experimental errors of various kinds, were on the whole about the same at all stages. Exposure to four degrees of frost had no apparent effect. The effect of heavier frost on the gl ıten yields of the very immature samples was masked of course by the blending procedure used. Even so, the yield in most of these cases was less than that of the corresponding check or 4° samples. The ratio of dry gluten to the crude protein content of the flour, shown in the last three columns of the table, was in all but a few cases, again chiefly among the very immature, heavily frozen samples, a little greater than unity.

The lowest score for texture and tenacity awarded any check gluten was 6, and there was gradual improvement from the earliest cuttings to those made at about 57–58% dry matter. The admixture of green kernels in the grain samples from the north end of the field is reflected in the lower quality of their gluten, as compared with that of the more uniform samples from the south end cut at corresponding dry matter contents. Immaturity had a

greater effect upon color than upon texture and tenacity, as seen in the steeper gradient of the color scores of the checks, from the earliest cuttings to those made at 57–58% dry matter.

It will be seen that even four degrees of frost damaged the gluten quality, color being affected up to the maturity represented by 56.8% dry matter, and texture and tenacity up to 57.3% dry matter, the last cutting exposed to this amount of frost. Heavier frost increased the amount of the injury and extended the range of susceptibility, which included, with respect to texture and tenacity, even the most mature samples subjected to 14 degrees of frost.

It must be concluded that the physical properties of the gluten are more easily affected by frost than any chemical property studied in the grain or flour. This of course is very important from the point of view of baking quality, a subject to be considered in the next paper.

Sugar Content and Diastatic Activity of Flour

The sugar content and diastatic activity of the flours milled from the frozen and check samples of wheat are given in Table XI. Diastatic activity is indicated by the maltose production of 10 gm. of flour digested for one hour under the conditions of the method already cited.

The reducing sugar made up little more than 10% of the total sugar. Both reducing and invert sugar percentages declined in the earlier check samples, but not so much as found by Brenchley and Hall (5) and Thatcher (30), who analyzed grain brought directly from the field and dried promptly in the laboratory. It has been shown earlier in this paper that respiration during slow drying, such as would take place in these samples cured in stooks, was sufficient to change appreciably the composition of the kernels, the greatest change occurring in the most immature samples (Fig. 2). No doubt much of the free sugar is used up in the process.

Four degrees of frost was insufficient to cause any consistent differences in the sugar content, but 8, 10 and 14 degrees led to increasing amounts of reducing sugar in flours from grain cut while containing less than 57–58% dry matter. There are no differences in the percentages of invert sugar which can be attributed to the freezing treatment of the grain.

If we make the assumption suggested when discussing the total nitrogen content of the frozen grain, that frost injury to the germs slows up respiration during subsequent drying, then we have at least one possible explanation why the frozen samples are higher in reducing sugar than the check samples, and why the differences from the checks increase with immaturity of the grain and severity of frost exposure. The laboratory germination percentages (to be reported elsewhere) of the grain samples from which these flours were milled, were reduced in the two most extreme cases from 100 and 99 in the checks to 9 and 16 in the frozen samples; and even these figures do not tell the whole story, as field tests showed the seedling vigor in the frozen samples

TABLE XI

SUGAR CONTENT AND DIASTATIC ACTIVITY (MALTOSE PRODUCTION) OF FLOUR MILLED FROM FROZEN AND CHECK SAMPLES OF WHEAT

Dry matter at cutting, %	Reducing sugar %			Invert sugar %			Maltose produced per hour, mg.		
	8° frost	4° frost	Check	8° frost	4° frost	Check	8° frost	4° frost	Check
31.2	1.29	0.92	0.94	6.56	6.09	—	245.5	212.0	—
34.9	0.96	0.59	0.69	5.33	5.23	5.51	253.8	186.8	160.8
39.4	0.92	0.67	0.58	4.28	4.42	4.42	197.7	136.1	132.2
43.9	0.72	0.63	0.52	3.57	3.98	4.04	279.7	202.6	154.0
46.8	0.51	0.50	0.52	3.60	3.81	3.75	239.2	185.2	180.1
48.3*	0.70	0.74	0.72	3.96	3.90	3.92	270.0	198.8	181.7
50.5*	1.01	0.69	0.98	4.05	3.68	3.98	316.8	227.2	151.9
51.1*	0.82	—	0.55	3.78	—	3.19	226.0	—	168.0
51.3*	0.78	0.53	0.62	3.44	3.90	3.81	218.6	239.4	221.5
55.8*	0.52	0.48	0.45	3.25	3.04	3.50	423.9	340.7	174.9
56.8	0.66	0.64	0.58	3.51	3.90	4.12	233.8	142.9	122.4
57.3	0.55	0.44	0.46	3.60	3.66	3.90	154.4	132.4	135.3
58.1*	0.53	—	0.51	3.20	—	3.52	137.9	—	154.4
59.1*	0.53	—	0.51	3.00	—	3.28	193.2	—	119.0
65.5*	0.57	—	0.54	3.36	—	3.34	105.4	—	157.4
65.6	0.51	—	0.48	3.35	—	3.38	148.0	—	97.2
	14° frost	10° frost	Check	14° frost	10° frost	Check	14° frost	10° frost	Check
34.0	—	—	0.94	—	—	6.05	—	—	155.3
38.1	2.16	1.85	0.61	4.14	3.39	5.21	443.9	434.2	131.6
40.9	1.11	1.04	0.59	3.80	3.65	3.57	453.2	503.0	179.7
45.5	0.91	0.65	0.63	3.74	3.44	3.64	419.7	189.7	145.8
46.7	0.71	0.66	0.54	3.40	2.88	3.46	472.4	243.0	165.8
50.5*	1.22	0.89	0.76	3.81	3.40	3.80	280.8	316.7	187.4
51.1*	1.15	—	0.55	4.30	—	3.19	348.6	—	168.0
53.3*	0.91	0.74	0.49	3.52	3.01	3.28	385.4	348.5	199.6
54.9	0.82	0.57	0.45	3.42	3.42	3.50	241.8	226.4	171.3
57.4*	0.53	0.46	0.49	3.19	2.85	3.46	245.4	250.4	161.2
58.1*	0.55	—	0.51	3.31	—	3.52	219.0	—	154.4
58.5	0.49	0.52	0.51	3.19	3.53	3.59	151.2	113.8	105.4
59.1*	0.56	—	0.51	3.24	—	3.28	205.8	—	119.0
65.5*	0.53	—	0.54	3.29	—	3.34	145.9	—	157.4
65.6	0.49	—	0.48	3.25	—	3.38	166.7	—	97.2
69.4	—	—	0.45	—	—	3.42	—	—	122.7

**Samples from north end of field.*

to have been very much impaired. If we assume respiration to be a characteristic of living cells and to vary with vital activity, the other assumption would seem to follow.

On the other hand, Bailey and Gurjar (2) reported the respiratory activity of frosted wheat to be greater than that of sound wheat. Their conclusion, however, is based on "two samples of commercial wheat containing frosted kernels." Since samples of this description frequently originate in fields caught by night frost while immature, it seems possible that immaturity rather than frost explained their results. In any event, some of the data

obtained in the present study accord better with the hypothesis that frost injury retards respiration, and pending a more critical experiment there does not seem sufficient warrant to reject it.

Bailey and Gurjar's suggestion that the disorganization of the protoplasm by frost sets free hydrolytic enzymes and leads to an accumulation of split products, such as dextrose, may be readily accepted. Our own figures for diastatic activity, recorded in the last three columns of Table XI, though erratic, show in general that this property declined with maturity and that frost increased it decidedly, even four degrees having a noticeable effect, and heavier exposures proportionately more. The frost effects appeared to persist to full maturity, though they were of relatively minor importance in samples cut at 58% dry matter or later.

Part of the reducing sugar accumulation noted in the flour samples from wheats frozen while immature may reasonably be attributed, therefore, to accelerated enzyme activity in the kernels after thawing. This explanation is, however, inapplicable to the effect of frost on the per cent total nitrogen in the grain, noted earlier.

Translocation and Respiration after Cutting

The higher weights of nitrogen and dry matter per 1,000 kernels in the checks as compared with the heavily frozen samples, among those cut while immature, suggested that translocation from the vegetative parts to the kernels must have taken place in the former after cutting and stooking. In the left-hand section of Table XII, these values are given for the first seven

TABLE XII

TRANSLOCATION AND RESPIRATION AFTER CUTTING IN RELATION TO STAGE OF MATURITY AND EXPOSURE TO FROST

Dry matter at cutting, %	Actual data						Calculated data				
	Weight per 1,000 kernels, gm.			Weight of protein per 1,000 kernels, gm.			Theoretical weight[a] per 1,000 kernels, gm.		Respiration losses[b] per 1,000 kernels, gm.		Gross gains[c] of checks by translocation, gm.
	Frozen	Check	Net gain by translocation	Frozen	Check	Net gain by translocation	Frozen	Check	Frozen	Check	
34.0	12.48	13.41	0.93	1.949	2.280	0.331	13.9	16.3	1.4	2.9	3.8
38.1	14.60	16.00	1.40	2.252	2.736	0.484	15.9	19.3	1.3	3.3	4.7
40.9	18.33	19.20	0.87	2.776	3.129	0.353	19.5	22.0	1.2	2.8	3.7
45.5	20.71	21.97	1.26	3.112	3.454	0.342	21.8	24.1	1.1	2.2	3.5
46.7	23.28	23.34	0.06	3.602	3.739	0.137	25.0	26.0	1.7	2.6	2.7
50.5*	22.78	22.78	0.00	3.329	3.443	0.114	22.6	23.4	−0.2	0.6	0.6
53.3*	24.95	24.82	−0.13	3.676	3.722	0.046	24.5	24.8	−0.4	0.0	−0.1

*Samples from north end of field.

[a] *Calculated from actual weights of protein in frozen and check samples, and percentages of protein in samples of maturity series oven-dried directly.*

[b] *Differences between actual and theoretical weights per 1,000 kernels.*

[c] *Sums of actual net gains in weight per 1,000 kernels by translocation and of calculated losses by respiration.*

cuttings exposed to 10 and 14 degrees of frost, averaged for the two frozen samples in each cutting, and with the nitrogen expressed as crude protein (N × 5.7). The apparent net gains of the checks by translocation, being the differences between the figures for frozen and check samples given in columns 4 and 7, are quite substantial in the first four cuttings, after which they diminish rapidly to negligible proportions. To the extent that translocation was not stopped by the frost, these differences would be proportionately too small, but it will be shown below that translocation after freezing was probably negligible.

On the other hand, it is quite clear from the nitrogen percentage relations in the earlier cuttings that respiration occurred in both frozen and check samples after stooking. The nitrogen percentages in the heavily frozen samples are of the order of those found in the maturity series in samples air-dried in the laboratory, while those of the checks and lightly frozen ones are still higher. The lowest percentages of nitrogen were found of course in the samples oven-dried directly. These differences appear to be explainable only on the basis of different amounts of carbon loss by respiration after cutting, following naturally from the different rates of drying these various samples, modified in certain cases by the freezing treatments.

To get some idea of the order of these respiration losses, the calculations reported in the right-hand side of Table XII were resorted to. If we accept the percentages of nitrogen found in the maturity series in the samples oven-dried directly, as representing the true ratio of protein to dry matter at a given stage of maturity, and assume that the departures from this ratio in other samples cut at corresponding stages are due to respiration after cutting, then the elimination of this respiration would lead to the theoretical weights per 1,000 kernels indicated in columns 8 and 9, from which can be deduced the respiration losses given in the next two columns. Calculated this way, the loss from the frozen samples was about half that from the check samples.

The theoretical weights of the frozen samples are of about the same order as the actual weights of the samples in the maturity series cut at the same stages of maturity (found by graphic interpolation of the values in Table I) and oven-dried directly. The first four may be compared as follows:

Frozen	13.9	15.9	19.5	21.8
Dried directly	13.4	16.2	18.3	21.7

It may be concluded, therefore, that the freezing treatment, while it reduced respiration only to about half the intensity found in the checks, reduced translocation to negligible proportions. We are justified then in the case of the checks to add to their actual net gain by translocation the amount of their loss by respiration, to get their gross gain by translocation after cutting, as given in the last column of the table.

Respiration of course continues in the fully matured grain as long as it remains alive, but the method employed here, subject as it is to sampling and other experimental errors and depending upon assumptions which can be

regarded only as approximations, is too rough to show consistent losses except in the more immature samples. The negative quantities occurring among the values for the last two samples in the table are evidences of the errors involved, though it is less surprising to find them attached to samples from the north end of the field, which have given somewhat erratic results throughout all these experiments, and are moreover not strictly comparable with samples of the maturity series, all of which came from the south end. The data appear to justify the conclusion that respiration losses after cutting are of substantial size in grain cut at a dry matter content of less than 50%.

Translocation after cutting must stop earlier than respiration, but all we can conclude from these results is that it probably is substantial only in samples with less than 50% dry matter at cutting. This relation of maturity to translocation after cutting is in harmony with the results of the joint experiment by Saunders (23) and Shutt (27), cited at the outset of this paper. The failure of Arny and Sun (1), Wilson and Raleigh (33) and Greaney (10) to detect in the weights of kernels cut at different stages of maturity any evidence of translocation after cutting immaturely, may be laid at least in part to their failure to allow for respiration losses, which under the conditions of the present experiment appear to offset about two-thirds of the gain by translocation.

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THE MICRO-ORGANISMS IN PROFILES OF CERTAIN VIRGIN SOILS IN MANITOBA[1]

By M. I. Timonin[2]

Abstract

Twelve profiles of five different kinds of virgin soils of Manitoba were sampled, described and critically examined for soil organisms, hydrogen ion concentration and the moisture and organic matter present. Usually the A horizon showed the highest count of each group of micro-organisms and the C horizon the lowest, although the greatest number of bacteria were present in the B horizon of Soil 1 in the month of May. The proportion of anaerobic bacteria and fungi to total numbers increased with the depth of the horizon. Moisture content of the soil was not found to exert any consistent effect upon the numbers of micro-organisms present. Fungi were most abundant in the wooded and peat soils, bacteria more so in soils of the meadow-prairie phase. In the wooded soils the microbiological horizons appeared to coincide with the morphological horizons.

Introduction

The physical and chemical features of various horizons in typical soil profiles have been considered by many investigators, but little information in relation to the soil organisms is on record.

This work represents a continuation of a previous study (4) on the identity, prevalence and significance of fungi found in Manitoba surface soils. It covers detailed investigation into the micro-organisms, moisture, organic matter and pH reaction of the various horizons in 12 profiles representing five distinct virgin soils of Manitoba.

Brown and Benton (7) made microbiological studies of some typical Iowa soil profiles. They report the largest number of bacteria and actinomycetes in the A_1 or A_2 and the largest number of fungi at times in the A_3 sub-horizons. The most striking decreases occurred from the A_3 to the B sub-horizons, although further decreases in each successive sub-horizon were noted. Fungi decreased in numbers relatively less than actinomycetes, and actinomycetes less than bacteria. Variation in moisture content seemed to have no definite relation to the number of micro-organisms, especially in lower horizons. A seasonal effect on numbers was noted although this was confined chiefly to the A_1, A_2 and A_3 sub-horizons. In some cases soil type differences were reflected by the number of micro-organisms in the various horizons. Organic matter, as noted by color, was observed to be the one significant characteristic influencing quantitative occurrence of organisms. Little relation existed between variations in conditions in the subsoil and counts of micro-organisms, although more bacteria and actinomycetes were found in the subsoils of loess type than in those of drift origin.

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This and part of the succeeding paper formed a thesis submitted to the Committee on Graduate Studies, University of Manitoba, in partial fulfilment of the requirements for the degree of Master of Science.

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McKibbin and Gray (13), studying chemical and microbiological factors in Quebec soils, noted that microbiological activity is dependent to some extent on the amount of water present in the soil. They considered that soil type has a greater influence on the numbers of micro-organisms than season. Later Gray and McMaster (11), in a study of podsol soils, submitted evidence in support of the view that numbers of bacteria as well as microbiological activity, as measured by carbon dioxide production and ammonification of urea, appear to be influenced by organic matter relations of well differentiated horizons.

Newton (14) reported that the highest bacterial count was obtained in Alberta soils in the spring. Waksman and Purvis (18) determined the numbers of micro-organisms in Highmoor and Lowmoor peat profiles in New Jersey. In their conclusions they noted that the sphagnum layer of Highmoor soils contains relatively few bacteria, but below the sphagnum layer, or as soon as the sedimentary layers of the peat are reached, there is a great increase in the number of bacteria. In the case of fungi a rapid decrease in numbers with increasing depth was noted.

Several workers have reported on micro-organisms at different depths in the soil, without correlating depth and soil horizon.

Methods of Sampling and Analyzing

A pit was dug in a representative area of each soil and blocks of soil about 8 in. (20 cm.) square were cut from the vertical side, showing characteristic horizons. The successive layers of the profile thus obtained were wrapped in heavy paper for transportation to the laboratory. Field notes recording the depth of the various horizons and their characteristics, temperature of each horizon, root development and surface flora were taken. In the laboratory a portion was removed from each horizon for moisture determination and the remainder of the block was rewrapped and held in a cool basement until the following day, when microbiological analyses were made on a moisture-free basis, using standard bacteriological technique for diluting and counting (5, 10).

The percentage of soil organic matter was determined by Tiurin's volumetric method, using Ischerekoff's coefficient for organic matter (17). The method was modified slightly: 25 ml. of 0.2 *N* chromic acid was used instead of 50 ml. After boiling, the solution was made up to 250 ml. with distilled water. One hundred ml. of the latter was titrated against Mohre's solution. The results were multiplied by 2.5 and the percentage expressed on the moisture free basis.

The hydrogen ion concentration was measured by a Leeds and Northrup potentiometer, using a quinhydrone electrode with a saturated calomel cell (2, 3). The moisture equivalent was determined by the method outlined by Briggs and McLane (6) and the hygroscopic and wilting coefficients calculated by Alway and Russel's method (1).

The soils reported on in this investigation are classified and described according to Nikiforoff's scheme of soil classification which was modified by Ellis for Manitoba soil surveys (9). They represent five different virgin soils.

Soil I

Profile 1

Profile 1 was taken from the meadow-prairie phase of the Fort Garry association in the Red River Valley combination in the chernozem zone. The area sampled is located at 49° 48′ north latitude and 97° 10′ west longitude, about three miles west of the Pembina highway and two miles south of the city of Winnipeg. The parent material is alluvium and shallow lake deposit laid over the deep laminated lacustrian clay of Lake Agassiz.

The topography of the area is level and the precipitation water collects in the low-lying areas. The micro-relief of the region is hummocky, the hummocks being 3–5 ft. in diameter and 4–6 in. high. The pit for sampling was dug through the diameter of the hummock and the profile samples were obtained below the centre of the hummock.

Ellis and Shafer (8) have reported on the prevailing climatic conditions in this area in relation to soil phenomena, presenting the monthly rainfall and maximum, minimum and mean monthly temperatures for each growing season at Winnipeg for more than 50 years previous to 1928. The averages of the mean monthly temperatures during the growing season for the 53 year period 1875–1927 were as follows: April 38.01; May 51.98; June 62.17; July 66.43; August 63.53; September 53.97; and October 41.01° Fahrenheit. The winter precipitation, principally snow, is light, and has varied in the period 1875–1927 from 1.96 inches (recorded as water) in 1921 to 8.75 inches in 1882. The mean rainfall for April to October for the period 1872 to 1927 was 16.15 inches. For the year 1933 the monthly precipitation and maximum, minimum, and mean temperatures at Winnipeg are shown in Table I.

TABLE I

Monthly precipitation and maximum, minimum, and mean temperatures at Winnipeg, 1933

Month	Temperature in deg. Fahrenheit			Precipitation in inches		
	Max.	Min.	Mean	Snow	Rain	Total
January	33.4	−30.6	2.3	10.4	—	1.04
February	39.1	−42.0	−1.9	6.1	—	0.61
March	48.4	−18.8	17.1	2.7	0.20	0.47
April	72.2	13.8	36.9	7.8	0.20	0.98
May	84.0	27.0	54.7	—	5.27	5.27
June	96.8	35.0	68.0	—	0.97	0.97
July	91.2	46.6	69.1	—	1.61	1.61
August	93.2	38.2	66.7	—	3.63	3.63
September	79.0	31.8	56.6	—	2.69	2.69
October	72.8	13.6	36.3	6.6	0.01	0.67
November	40.2	−16.2	17.5	17.2	—	1.72
December	35.0	−42.4	−4.6	15.7	—	1.57
Total				66.5	14.58	21.23

The vegetative cover consists of a solid sod composed of the following grasses, *Poa pratensis*, *P. compressa*, *Agropyron repens* and *A. tenerum* but mixed with *Grindelia squarrosa*, *Argentina anserina*, *Aster multiflorus*, species of *Antennaria* and *Solidago*. *Salix* sp., *Symphoricarpos occidentalis*, and *Populus tremuloides* dot the surface in low-lying areas.

Ants were active on some of the hummocks.

Description of Profile 1. A_1 (0–2.5 in.) consists of a well decomposed dark brown organic material mixed with black silty clay, and a well developed mat of roots. This sub-horizon can be easily separated from A_2.

A_2 (2.5–5 in.) consists of black clay in closely packed, hard, dull-faced granules.

B_1 (5–11 in.) is a silty clay, gray in color, streaked vertically with darker colors. The horizon has a weakly developed structure, being crumbly, friable and highly calcareous. Sub-horizon B_1 slowly grades into B_2; the line of demarcation is hard to discern. Roots are not as abundant as in A_2.

B_2 (11–19 in.) is a very fine sandy clay, buff colored, crumbling easily. Specks of iron concretion are common. Root proliferation is slight. Sub-horizon B_2 is hard to separate from the parent material.

C (19–31 in.) is a light buff colored clay and very fine sand, with thin layers of gray clay richly speckled with iron accumulation. Pinholes of decayed roots are quite numerous.

This profile represents normal meadow-prairie soil. The tongued intrusions at the margins of the hummocks are due to the cracking of the soil, which occurs during dry spells, and of intrusion of surface soil through these cracks into the subsoil (8).

Profile 2 is a duplicate of Profile 1, obtained 13 days later (May 15) by removing the loose earth and cutting back the face of the pit about 1 ft. before sampling.

Profile 3, taken on June 20, represents the same type of soil as Profile 1. It was located in the zone of transition of the Red River Valley association into the Fort Garry association. Islands of alkalinized Red River and Fort Garry meadow prairie soils are intermixed in this area.

The location of Profile 3 is about two miles southeast of the area of Profile 1, about 100 yards west of Pembina highway and about 300 yards northeast of the electric power line. The surface of this area is less hummocky and the hummocks are larger in diameter and lower in height. Vegetation is apparently the same as in Profile 1.

Profile 4 is a duplicate of Profile 1. The pit was dug about 25 yards northeast of Profile 1. The samples were obtained September 21.

The results of microbiological and chemical analyses of Profiles 1 to 4 are shown in Table II.

TABLE II

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL I

Profile no.	Date of sample	Depth of horizons, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio†	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi*	Algae*	Protozoa*
1	May 2, 1933	A_1(0–2.5)	9	37.9	45.42	0.83	7.31	6.68	8.04	49,150,000	4,800,000	1,000,000	26,000	1,000	–
		A_2(2.5–5)	7	27.7	37.27	0.74	8.35	7.26	3.18	131,800,000	2,200,000	1,000,000	2,167	5,000	–
		B_1(5–11)	5	30.5	34.53	0.88	8.27	7.45	2.41	158,300,000	700,000	10,000,000	320	0	–
		B_2(11–19)	3	29.3	32.82	0.89	8.24	7.54	1.76	45,300,000	700,000	1,000,000	220	0	–
		C (19–31)	0	24.9	19.70	1.26	8.16	7.69	0.86	6,000,000	0	1,000	90	0	–
2	May 15, 1933	A_1(0–2.5)	17	25.6	52.39	0.49	7.71	6.81	19.44	37,830,000	2,430,000	–	28,166	10,000	–
		A_2(2.5–5)	10	27.1	39.99	0.68	7.95	6.84	5.81	124,266,000	2,000,000	–	3,000	5,000	–
		B_1(5–12.5)	6	31.6	38.60	0.82	8.11	7.24	2.36	147,066,000	600,000	–	388	500	–
		B_2(12.5–20)	3	31.1	31.94	0.97	8.07	7.38	1.22	33,380,000	620,000	–	220	40	–
		C (20–27)	0	21.3	21.14	1.01	8.01	7.75	0.77	6,033,000	0	–	40	0	–
3	June 20, 1933	A_1(0–1.5)	27	14.8	40.37	0.37	8.03	7.00	8.96	19,200,000	3,825,000	1,000,000	46,750	10,000	40
		A_2(1.5–4.5)	24	24.2	41.00	0.59	8.08	6.98	6.54	26,525,000	4,125,000	100,000	40,500	1,000	20
		B_1(4.5–8.5)	21	16.2	39.95	0.41	8.12	7.20	2.44	4,700,000	400,000	100,000	1,000	0	0
		B_2(8.5–17.5)	20	22.3	37.90	0.59	8.29	7.44	1.56	2,310,000	77,000	100,000	632	0	0
		C (17.5–38)	17	20.0	23.22	0.86	8.12	7.52	0.87	217,000	0	1,000	98	0	0
4	Sept. 21, 1933	A_1(0–2)	12	82.0	53.67	1.52	7.46	6.75	22.25	19,000,000	3,250,000	1,000,000	60,127	1,000	100
		A_2(2–6)	11	24.3	32.06	0.76	8.08	6.83	8.64	16,500,000	3,000,000	1,000,000	6,000	500	20
		B_1(6–16)	12	31.7	41.72	0.76	8.09	6.80	2.45	16,725,000	650,000	1,000,000	2,500	0	0
		B_2(16–27)	14	31.7	33.87	0.94	8.25	7.31	1.27	2,513,000	152,000	1,000	200	0	0
		C (27–44)	13	18.8	19.17	0.95	8.27	7.49	0.31	280,000	0	1,000	40	0	0

†*Moisture ratio* $= \frac{\textit{Total moisture}}{\textit{Moisture equivalent}}$; * = *Number of organisms per gm. of moisture-free soil;* – = *No data available.*

Soil II

One profile, No. 5, was taken from Soil II, about 50 yards north of Profile 3. This soil differs from Soil I only in being an alkalinized phase instead of a meadow-prairie phase. The profile is characterized by a granular structure in the upper part of the poorly defined B horizon, and by the formation of columns about 7 × 3 in. in the grayish brown clay of the lower part of the B and the upper part of the C horizons (10 to 40 in. in depth). The counts of micro-organisms in Soil II were similar to those in Soil I. Details are not given in this paper, but the species of fungi isolated are dealt with in the paper immediately following this.

Soil III

Profile 6 was taken June 29 from the wooded phase of the phytomorphic associate of the Fort Garry association in the Red River Valley combination in the chernozem zone.

The location of the profile is about six miles north of Profile 1 and about one mile west of the Assiniboine river.

The vegetation consists chiefly of poplars, *Populus tremuloides* and *P. balsamifera*, with clumps of oaks, *Quercus macrocarpa*, (up to about seven inches in diameter) and *Corylus americana*, *C. rostrata*, *Viburnum pubescens*, *Crataegus coccinea*, *Symphoricarpos occidentalis*, *Pyrola asarifolia*, *Aralia nudicaulis*, *Carex stenophylla* and *Unifolium canadense*.

Earthworms were observed frequently.

Description of Profile 6. A_0 (0–1¼ in.): leaf mat well decomposed, penetrated by mycelium and rhizomorphs of fungi.

A_1 (1¼–3½ in.): dark fine sandy clay, powdery, with buckwheat-sized granules, mixed with finely divided organic material and penetrated by numerous roots.

A_2 (3½–7 in.): whitish-gray, heavy, very fine sandy loam with aggregations of granules on which specks of silica can be seen. Roots penetrate this horizon vertically.

B (7–17 in.): coffee-brown silty clay, shading to a lighter color in the lower part of the horizon. The structure is coarsely granular, breaking into fine granules. It is quite compact with a finer texture than A_2. Root development is more profuse than in A_2.

C (17–36 in.): light buff colored clay with very fine sand. It is the same material as in horizon C of Profile 1.

This profile represents a slightly podsolized soil. The horizons are in the process of formation and are gradually assuming the features of podsol soils.

Profile 7 represents the same soil as Profile 6. It was taken about one-half mile north of Profile 6. The surface vegetation is practically the same. The samples were obtained July 10.

Profile 8 is a duplicate of Profile 6 made by removing the loose earth from the original pit and cutting back one face about a foot before sampling. The samples were taken August 28.

The results of microbiological and chemical analyses of Soil III are shown in Table III.

Soil IV

Profile 9 was taken from a podsolic soil area developed from an alluvial deposit. It was sampled on July 19 and plated July 20.

The sampled area is located about 50° 15′ north latitude and 96° 03′ west longitude, at the southern limits of the town of Lac du Bonnet and about 300 yards from the shore of Lac du Bonnet. The average temperature is slightly lower and the precipitation slightly higher than at Winnipeg, although 1933 was exceptionally dry at Lac du Bonnet.

The topography of the region is rolling but the area where the samples were obtained is more of a plain sloping towards the lake. This area is heavily wooded and has considerable underbrush. The vegetation of this area consists principally of the following: trees, *Populus tremuloides*, *Betula* sp., *Fraxinus nigra*, *F. pennsylvanica* and a few *Pinus resinosa;* shrubs, *Amelanchier alnifolia*, *Rhamnus alnifolia*, *Cornus stolonifera*, *Acer spicatum*, *Viburnum opulus*, *Ribes americana*, *Lonicera oblongifolia*, *Rubus triflorus*, *Corylus americana*, *C. rostrata;* and herbs, *Aralia nudicaulis*, *Geum strictum*, *Unifolium canadense*, *Smilacina stellata*, *Mitella nuda*, *Aster* spp., *Lappula echinata*.

Description of Profile 9. A_0 (0–4 in.): undecomposed and partly decomposed forest debris forming a well defined mat.

A_1 (4–5 in.): very thin and poorly developed; composed of silty clay mixed with organic debris, finely granular and dark colored.

A_2 (5–8 in.): whitish-gray fine sandy loam with indistinct line of demarcation from the overlying sub-horizon A_1, but quite distinct from the underlying horizon B. It has a platy structure which crushes into an ash-like powder; no visible organic matter.

B (8–19 in.): dark olive-brown silty clay with a finely nutty structure, aggregated into larger clumps an inch or so in diameter; compact and heavier in texture than A_2. Roots are developed very well in this horizon, and a few earthworms were observed.

C (19–37 in. and deeper): alluvial deposit of clay and very fine sand, light brown with accumulation of calcium. Structure is friable and coarser in texture than horizon B.

Profile 10 is of the same soil as Profile 9 and was obtained about one half mile from the lake and about two miles south of the town. It was sampled July 19, and the wrapped samples were held in a cool basement until July 31

TABLE III

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL III

Profile no.	Date of sample	Depth of horizons, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi*	Anaerobic fungi*	Fungi at 37°C.*	Algae*	Protozoa*
6	June 29, 1933	A_0 (0–1.5)	18	99.0	108.69	0.91	7.39	6.86	60.78	1,000,000	122,500	100,000	117,000	1,120	450	1,000	40
		A_1 (1.5–3.5)	17	36.8	62.19	0.59	6.77	5.57	27.01	30,000,000	7,500,000	1,000,000	15,625	1,010	100	1,000	10
		A_2 (3.5–7)	15	14.4	25.97	0.55	6.36	5.36	3.52	1,835,000	85,000	1,000,000	4,250	590	40	20	0
		B (7–17)	14	23.9	44.27	0.54	7.14	5.97	2.12	4,300,000	950,000	1,000,000	995	540	0	0	0
		C (17–36)	12	18.5	22.44	0.82	8.16	7.54	0.35	205,000	4,000	10,000	120	50	0	0	0
7	July 10, 1933	A_0 (0–2.5)	20	60.6	127.86	0.47	7.41	6.92	61.34	10,650,000	4,350,000	1,000,000	224,375	330	–	1,000	40
		A_1 (2.5–3.5)	18	17.8	27.55	0.65	7.21	6.61	19.18	31,000,000	9,000,000	1,000,000	26,875	540	–	1,000	20
		A_2 (3.5–10)	16	15.2	24.39	0.62	6.67	5.53	3.44	7,150,000	1,550,000	10,000	30,125	500	–	0	0
		B (10–18)	15	21.1	27.65	0.76	7.20	5.99	1.59	10,600,000	1,800,000	1,000,000	2,450	40	–	0	0
		C (18–36)	14	19.0	33.21	0.57	8.16	7.31	0.66	389,000	19,000	10,000	110	20	–	0	0
8	August 28, 1933	A_0 (0–2)	14	83.3	75.05	1.11	7.31	6.82	40.37	12,850,000	2,700,000	1,000,000	345,000	1,380	100	5,000	100
		A_1 (2–3)	15	37.0	33.17	1.12	6.30	5.65	11.46	850,000	0	100	3,500	40	0	0	10
		A_2 (3–7.5)	15	16.9	25.55	0.66	6.50	5.82	3.13	5,195,000	1,905,000	15,300	10,000	380	10	20	0
		B (7.5–19)	15	20.7	36.81	0.56	7.30	5.96	3.38	13,500,000	2,500,000	100,000	16,400	330	0	0	0
		C (19–36)	14	12.1	13.64	0.89	7.95	7.16	0.32	710,000	60,000	1,000	550	30	0	0	0

* = *Number of organisms per gram of moisture-free soil.*
– = *No data available.*

before plating. The description of the profile is the same as that of Profile 9, except that the B horizon is more compact and the aggregations of granules are somewhat larger in size. The results are presented in Table IV.

Soil V

Profile 11 was taken from a peat soil, about three miles west of Pinawa Falls and five miles east from the shore of Lac du Bonnet. This peat was formed in a valley about one-half mile wide where there is no outlet for drainage water.

The vegetation of the valley area consists chiefly of the following: *Larix laricina*, *Picea canadensis*, *Betula glandulosa*, *Populus tremuloides*, *Chamaedaphne calyculata*, *Andromeda polifolia*, *Oxycoccus oxycoccus*, *Ledum groenlandicum*, *Geum strictum*, *Equisetum* spp., *Carex* spp., and *Aster* spp. The plants mentioned above grow amongst *Sphagnum* moss, together with some *Polytrichum* moss.

A pit for sampling was dug down to the heavy blue clay and samples were taken at three depths.

Description of Profile 11. 1 (0–7 in.). The surface layer is a growing sphagnum layer which varies considerably in depth.

2 (7–35 in.). The second layer is a felted moss layer intermixed with branches of trees. Waterlogging begins at 20 in. and water gradually increases with the depth. The sample was taken at a depth of from 27 to 35 in.

3 (58–64 in.) Well decomposed remains of the sphagnum moss are mixed with sticks and limbs of trees, the bark of which is decomposed. An odor of hydrogen sulphide is noticeable. Below 64 in. is a heavy blue clay layer.

Profile 12 represents the same area as Profile 11. The pit was dug about 100 yards south and across the road from Profile 11. The morphological and organic characteristics are the same as in Profile 11.

The results are given in Table V.

Results of Studies

The numbers of bacteria and actinomycetes were highest in the second or third depths, except in peat and in Profile 10, where the first depth gave the highest count, and in Profile 8, where the B horizon was highest. The counts of bacteria and actinomycetes in the B_2 sub-horizons of Soil I were similar to the counts in the B horizons of Soils III and IV, being in all cases, except Profile 8, between 11.7 and 31.5% of the highest counts in the profiles. The counts in the C horizons were uniformly low.

The counts of actinomycetes ranged from 0 to 43.7% of the total number of micro-organisms counted on Brown's sodium albuminate agar (10). They were more abundant in one of the two surface sub-horizons in all profiles. There was a marked decrease in the B horizons in the prairie soils, which was

TABLE IV

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL IV

Profile no.	Date of sample	Depth of horizons, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi*	Anaerobic fungi*	Fungi at 37°C.*	Algae*	Protozoa*
9	July 19, 1933	A_2 (0–4)	17	56.9	132.17	0.43	6.39	6.01	91.92	16,915,000	1,335,000	1,000,000	205,000	1,326	400	500	40
		A_1 (4–5)	15	27.1	69.68	0.39	6.21	5.45	77.02	73,000,000	16,000,000	10,000,000	65,000	970	195	5,000	20
		A_2 (5–8)	14	14.1	29.65	0.48	5.94	5.17	17.90	7,650,000	950,000	10,000	7,500	1,650	0	100	0
		B (8–19)	11	21.4	40.28	0.53	7.38	6.24	17.26	9,750,000	1,250,000	100,000	14,750	2,380	0	100	0
		C (19–37)	11	19.4	30.68	0.63	7.94	7.18	0.63	463,000	97,000	10,000	1,840	680	0	0	0
10	July 19, 1933	A_2 (0–3.5)	17	48.7	122.96	0.40	6.55	5.83	64.17	28,300,000	5,700,000	100,000	242,500	2,850	105	1,000	100
		A_1 (3.5–5)	15	23.1	71.52	0.32	5.51	4.99	25.00	4,500,000	3,500,000	1,000,000	20,000	2,650	100	100	20
		A_2 (5–8)	14	13.6	29.39	0.46	5.50	4.64	4.47	1,550,000	950,000	10,000	1,625	880	20	0	0
		B (8–23)	11	19.1	33.68	0.57	6.37	5.95	2.60	3,300,000	900,000	100,000	10,625	2,640	0	0	0
		C (23–37)	11	18.7	35.74	0.52	7.81	7.06	0.95	1,142,000	120,000	10,000	1,470	1,100	0	0	0

* = *Number of organisms per gm. of moisture-free soil.*

TABLE V

PHYSICO-CHEMICAL AND MICROBIOLOGICAL ANALYSES OF SOIL V

Profile no.	Date of sample	Depth of layers, in.	Temperature in °C.	Total moisture, %	Moisture equivalent	Moisture ratio	pH in H_2O	pH in KCl	Organic matter, %	Bacteria*	Actinomycetes*	Anaerobic bacteria*	Fungi	Anaerobic fungi*	Fungi at 37° C.*	Algae*	Protozoa*
11	July 19, 1933	1(0–7)	17	500.7	220.95	2.27	5.35	4.19	85.75	3,600,000	1,000,000	100,000	168,300	35,800	4,050	10,000	10
		2(27–35)	12	620.2	224.37	2.76	5.47	4.93	91.55	1,050,000	200,000	100,000	1,625	470	0	500	0
		3(58–64)	9	750	251.38	2.98	5.68	5.00	98.31	48,000	0	100,000	500	70	0	20	0
12	July, 19 1933	1(0–18)	17	500.7	190.22	2.63	4.78	3.86	90.24	2,900,000	1,150,000	100,000	372,500	30,950	1,075	10,000	10
		2(30–37)	12	620.2	188.92	3.28	5.43	4.92	68.00	2,740,000	10,000	100,000	3,100	530	0	500	0
		3(70–76)	9	750	232.91	3.22	4.39	4.68	88.91	63,500	0	100,000	875	50	0	0	0

* = *Number of organisms per gm. of moisture-free soil.*

less noticeable in the wooded soils. The prairie soils showed no growth of actinomycetes from the C horizons of four profiles, while the C horizon of the wooded soils gave counts ranging from 4,000 to 120,000 per gm. of soil.

Anaerobic bacteria varied in number throughout the profiles with the C horizons generally showing low counts.

The highest counts of fungi were found in the surface sub-horizons in all cases. The decreases in the numbers of fungi were most striking in the second sub-horizons. In the lower horizons the decreases were less marked. Even in the C horizons considerable numbers of fungi were found.

The number of fungi growing at 37° C. represented from 0–1.6% of the counts on plates incubated at 20° C. Growth at the higher temperature was confined to the fungi in the A_0, A_1 and A_2 sub-horizons.

Plates incubated in a carbon dioxide chamber yielded the same genera as those under aerobic conditions, but the colonies were not as numerous, varying from 0.6 to 78.6% of the counts on the plates incubated aerobically.

The algal flora is concentrated almost entirely in the A horizon.

Protozoa were found only in the two surface horizons. In the majority of cases ciliata only were observed. Amoebae were also found, however, only in the surface horizons in Profiles 4, 8, and 10.

Discussion of Results

In a review of the literature on the subject, one finds that most workers report that organic matter, moisture, hydrogen ion concentration, season and soil type are the factors which control the density of micro-organisms in the soil.

In this report the above mentioned factors are presented in Tables II, III, IV and V. In order to trace the influence of these factors on Manitoba soils, each will be discussed under a separate heading.

Organic Matter

There is no doubt that organic matter is the most important factor in the life of the majority of micro-organisms, because it is a source of food and energy.

Analyzing the results obtained, it is evident that the highest percentage of organic matter is found in the surface horizon in all profiles studied, except peat.

Comparing the number of micro-organisms with the recorded percentage of organic matter in the corresponding horizons which are morphologically well differentiated, the data seem to confirm the view expressed by others, that organic matter is the factor which controls the activity of the soil micro-organisms during the vegetative period. The subsoil horizons contain only a small fraction of organic matter as compared with the surface horizons and

also a correspondingly low fraction of the number of micro-organisms. Yet micro-organisms decrease with depth in peat, despite a high content of organic matter throughout.

In the wooded soils sub-horizon A_2, in all cases but one, showed a higher percentage of organic matter than horizon B. However, the total number of bacteria and actinomycetes in the wooded soils III and IV was higher in the B horizon than in A_2. In the case of fungi, a higher number was also recorded from horizon B than from A_2 in the wooded soils of Profiles 8, 9, and 10. In prairie soils a similar variation in the number of bacteria and actinomycetes occurred in the profiles which were sampled during May, but in the later samples the A horizon gave larger counts. Considering these facts, the conclusion may be drawn that organic matter is not the only factor influencing the microbiological population of soil horizons.

Moisture

An analysis of the data makes it evident that the total moisture content of the soils studied does not show the true picture of the soil moisture. For example, A_1 of Profile 1 contained 1.52 times as much moisture as horizon C of the same profile. However, the moisture equivalent shows that the A_1 sub-horizon can retain 45.42% of moisture against a pull of 1,000 times gravity exercised over a period of 40 min. On the other hand, horizon C retained only 19.7%. Also, the moisture ratio $\left(\frac{\text{T.M.}}{\text{M.Eq.}}\right)$ of horizon C is 1.58 times as great as that of sub-horizon A_1. That means that horizon C contains more available moisture for plant growth than A_1. For this reason it would be a mistake to take into consideration the total soil moisture as a factor influencing the microbiological population in soil horizons. The moisture ratio of the morphologically corresponding horizons of profiles obtained from the same soil at different dates shows no definite relation to the biological population of these horizons. Horizons in which the moisture ratio is below the wilting point may show higher numbers of micro-organisms than horizons which contain moisture available for plant growth.

Profile 8 of Soil III showed an abnormal distribution of micro-organisms in comparison with the other wooded soil profiles studied. The samples of Profile 8 were obtained August 28, three days after a heavy rain occurred. The rain started August 22 and continued until August 25. A total precipitation of 1.82 in. was recorded during the four days. Perhaps owing to this fact, sub-horizon A_1 gave the lowest counts of bacteria, actinomycetes and anaerobic bacteria and the second lowest of fungi in the profile. The pH was also lowest in sub-horizon A_1. The acidity of this sub-horizon was a limiting factor for bacteria and actinomycetes, but the fungi and algae should not be affected by this. However, the fungi were also much reduced in numbers. Accordingly, acidity can not be considered as the limiting factor. Possibly there is another soil factor acting as a soil disinfectant, but the data are too uncertain to warrant any conclusions.

Acidity

Thornton and Bear (15, pp. 398–400) obtained the highest counts of soil bacteria on agar at pH 7 and at slightly alkaline reactions. Russell (15, Chap. VI) states that the "general result is that fungi preponderate in acid soils, while bacteria become numerically much more important in neutral soils, but this is not because fungi thrive better in an acid than a neutral medium but because they tolerate acidity better than bacteria."

All the profiles of the wooded soils studied except Profile 8 just mentioned, showed the lowest pH in the A_2 sub-horizons. Owing probably to this fact, the numbers of bacteria and actinomycetes also were lower than in the B horizons. In prairie soils this phenomenon does not occur because all horizons are alkaline. In organic soils, Soil V in this case, the acidity of the soil is more pronounced than in the other soils studied, and the bacteria and actinomycetes lowest in numbers. From these results we may conclude that acidity is a factor lessening the number of bacteria and actinomycetes in the soil profile.

Season

A seasonal effect on the numbers of bacteria and fungi was reported by Brown and Benton (7) in Iowa soils. Conn (see 15, p. 401) claimed that freezing of soil increases the number of bacteria. Lochhead (12) found higher numbers of bacteria in frozen soil than at ordinary summer temperatures and much larger numbers of bacteria when the soil begins to thaw.

Table II shows that in Soil I the numbers of bacteria were higher in the May samples than in the June and September samples. It appears that in this soil, bacteria decrease with increasing soil temperatures. A decrease in the number of bacteria in the late season occurred in all horizons, including C. The numbers of fungi were altogether different. They were low in May samples, but increased in horizons A and B in September samples. The numbers of fungi in B_2 and C remained practically the same throughout the season. The data obtained indicate that with increasing soil temperature corresponding to the advance of the summer season, the number of bacteria decreases throughout all the horizons in the profile; a seasonal effect apparently occurs in all horizons of the soil profiles. On the contrary, the numbers of fungi nearer the surface increased as the soil temperatures increased. A seasonal effect on the fungi apparently does not occur in B_2 and C.

Soil Type

The numbers of micro-organisms obtained during June and July from the surface horizons of meadow-prairie, wooded and organic soil types, show that bacteria and actinomycetes in meadow-prairie soils are 1.6 to 20.5 times as great as in wooded and 5 to 5.7 times as great as in organic soil types. Fungi, however, are greater in number in wooded and organic than in meadow-prairie soils. The most noticeable characteristic in wooded soils is the striking increase of bacteria and actinomycetes from the A_0 to the A_1

sub-horizons, which was recorded from all profiles except 8 and 10. This exception in Profile 8 is perhaps due to excessive precipitation and in 10 is probably explained by the fact that the samples were kept for 12 days in the basement before plating. Sub-horizon A_2 in wooded soils is one from which substances are leached by percolating water and deposited in horizon B (15, Chap. IV). Owing to this fact the B horizon showed, in nearly all cases, a higher number of bacteria, actinomycetes and fungi than the A_2 horizon, even though the B horizons are heavier in texture and located deeper than the A_2 horizons. The numbers of micro-organisms in the well differentiated horizons of wooded soil profiles seem to be closely related to the morphological appearance of the horizons; in other words, in wooded soil the microbiological and morphological horizons are identical.

The numbers of protozoa obtained from virgin soils in Manitoba were very low, compared to those reported by Russell and others (15, pp. 403–410). Strelkov (16), studying degraded chernozem soils by Koffman's method, reported that no active forms were found in the surface soil. The quantitative protozoan population of the degraded chernozem soil was reported as follows: *Amoebae* 10–100 per gm., Flagellata 5,000–7,500 and Infusoria 10–100 per gm. Taking into consideration the above figures, the virgin soils of Manitoba showed a small population of protozoa.

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FUNGI ISOLATED FROM SOIL PROFILES IN MANITOBA[1]

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Abstract

Representative samples of the fungi in the soil horizons of 12 profiles of five types of virgin soil in Manitoba were identified. The isolations were made by the standard technique; and in addition to the routine method of incubating dilution plates aerobically at 25° C., other plates were incubated aerobically at 37° C., others aerobically at about 6° C., and others anaerobically at about 20° C. Fifty-six fungi not previously known in Manitoba soil were obtained. The more important species of fungi found in soil are discussed.

Introduction

In a previous paper (3) the writers reported on the fungi isolated from various surface soils in Manitoba, the majority of which were under cultivation, although a few were virgin forest or prairie. The present study deals with an investigation of the fungi in the various horizons of 12 profiles representing five different types of virgin soil. In a preceding paper, Timonin (20) presented descriptions of the soils studied, reported on the numbers of the various groups of micro-organisms found and showed the relations between numbers and types of micro-organisms and certain chemical and physical factors. This report includes the identification of the fungi isolated in this project and also a number not determined at the time the first paper (3) was presented. The list of soil fungi that follows includes only species not previously recorded from Manitoba soils.

Additional Fungi from Manitoba Soils

MYXOBACTERIALES

Myxococcus ?ruber Baur was found to be present in 24 of 32 isolations made from Profile 6. Drops of 1 : 10 dilution from sub-horizons A_1, A_2 and B_1 were placed on Ashby's mannitol phosphate agar (7). A few tests were made from other profiles and a yellow but undetermined member of the Myxobacteriales was found to be fairly common, and a pink species less common. These preliminary tests demonstrate that Myxobacteriales occur in Manitoba soils.

MYXOMYCETES

Myxomycetes occur commonly in Manitoba, and their spores fall in considerable numbers upon the ground. The few tests made with soil placed upon the mannitol phosphate agar (7), however, failed to disclose their presence.

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PHYCOMYCETES

Cunninghamella elegans Lendner. Four cultures of this striking member of the Mucorales were obtained from plates made for Myxomycetes from sub-horizon A_0 of Profile 6. The standard technique of isolation from soil failed to demonstrate the presence of this species.

Mortierella elasson Sideris and Paxton. A fungus which is this species or much like it was obtained frequently. The spores and sporangiophores are even smaller than described for *M. elasson*. The mycelium is persistently white, and in many isolations grows in the broadly zonate manner illustrated by Sideris and Paxton (18, Fig. 1). The hyphae usually are 4–6μ wide, but vary from 1 to 10μ, and are non-septate and vacuolate. Sporangiophores appear tardily or not at all on most media; on corn-meal agar they arise on the aerial mycelium, are usually simple although rarely branched, 30–100μ long and usually 4–8μ wide at the base and 2–3μ at the apex; sporangia are spherical, 8–20μ in diameter, the wall rupturing easily, leaving a slight turned down collar; the spores are oval to subspherical, mostly 2.5–4 $\times$ 3–6μ, thin walled, hyaline, with one or more guttulae; chlamydospores (gemmae) occur commonly on potato-sucrose agar and are 9–22μ in diameter. Different isolations vary somewhat but they seem to represent a single species near enough to *M. elasson* to be included with it, although possibly it deserves varietal rank. This form was found to be common in meadow soil, especially in the deeper horizons, and occasional in forest soil, alfalfa fields and elsewhere.

Mortierella vinacea Dixon-Stewart. This fungus, reported by Dixon-Stewart (6) from cultivated and bush soils in Australia, seems to be commonly present in forest and peat soils in Manitoba. The cultures usually are white at first, then often russet vinaceous as described, but there is considerable variation in the depth and shade of color in different isolations and on different media. The mycelium is irregular, mostly 4–8μ in diameter, but in older cultures bears many swellings (chlamydospores or gemmae) with guttulae. Sporangiophores may be branched or simple, and bear globose sporangia 15–50μ in diameter. Spores in sporangia are subglobose, 2–3μ in diameter, but swell greatly on the approach of germination and readily form germ tubes in water.

Mucor ?varians Povah. A few cultures, apparently of this species, were obtained from Soil IV.

Zygorhynchus vuilleminii Namys. This species was found a few times in the different horizons of Profile 7.

ASCOMYCETES

Chaetomium olivaceum Cke and Ell. (*C. globosum* Kunze). Five isolations of this fungus were obtained from surface soils.

Chaetomium setosum Wint. This species developed once in a culture from Horizon B incubated at 37° C.

Humarina ?convexula (Pers.) Seaver. A Discomycete with white to pale yellow apothecia, ascospores 12–16 × 8–10μ with two guttulae, and paraphyses thickened up to 16μ at the apex, developed in five cultures from Soil III incubated at 37° C.

?Pleospora sp. Two cultures from Soil IV produced perithecia and muriform spores.

Sclerotinia sp. One isolation from a surface soil was considered by Prof. Whetzel to be probably the haploid state of some *Sclerotinia*.

Xylaria sp. Three cultures from the surface of Soil I, incubated at 37° C. then transferred to flasks of leaves etc., developed striking *Xylaria*-like fruit bodies, but asci were not found.

Fungi Imperfecti

Acremoniella sp. A few cultures were obtained which produced dark terminal spores (chlamydospores?). These might be considered to belong to *Acremoniella* as considered by Saccardo, but their exact status is uncertain.

Acrostalagmus albus Preuss var. ***varius*** Jensen. Three cultures from Soil III.

Aspergillus nidulans (Eidam) Winter. One culture developed from the C horizon and one from a surface soil plate incubated at 37°.

Aspergillus sydowi (Bain. & Sart.) Thom and Church. One isolation from a C horizon.

Cephalosporium spp.? Several isolations produced heads of spores as in the genus *Cephalosporium*, but could not definitely be referred to described species. In some cultures the mycelium became dark. It is quite possible that some of these isolations represent "oidial" stages on haploid mycelia of Hymenomycetes or other fungi.

Coremium sp.? One isolation produced coremia, but it was not identified

Dematium sp. A few cultures could be placed only tentatively in this genus.

Epicoccum nigrum Link. Two or three cultures were obtained; Dr. J. E. Machacek finds it also in cereal roots. The dark spores are 20–25μ in diameter, coarsely spiny, on conidiophores somewhat penicillately branched.

Fusarium ossicolum (B. & C.) Sacc. = ***F. equiseti*** (Cda.) Sacc. Form 1 Wollenw. Isolated twice.

Fusarium oxysporum Schlecht. var. ***aurantiacum*** (Lk.) Wollenw. Three isolations from Soil I.

Geomyces vulgaris Traaen. Several isolations, particularly from Soil I, fit this species (21). Cultures varied considerably in color, but all seemed to fit *G. vulgaris* better than Traaen's other species of *Geomyces* (*G. sulphureus*, *G. auratus* and *G. cretaceus*). Traaen's work has been more or less overlooked. It may be remarked in passing that *Sporotrichum carnis* is quite similar to *Geomyces vulgaris*.

Haplographium bicolor Grove. Five isolations from the surface horizon of Soils I and IV, four of which developed from isolations at 37° C. The fungus agrees with Bunting's illustrations in Mason (11).

Haplographium fuscipes (Preuss) Sacc. One isolation from a forest soil. It is well illustrated by Bainier (1). Von Hoehnel (9, No. 89) reports Bainier's fungus to be a form of *H. penicilloides* Fautrey, which is the same as *H. finitimum* (Preuss) Sacc.

Helminthosporium geniculatum Tracy and Earle. Three isolations from horizon A of Soil I. All *Helminthosporia* were determined by Dr. J. E. Machacek.

Helminthosporium ?rhabdiferum Berk. & Br. (possibly a *Dendryphium*). Two isolations from surface soils.

Helminthosporium tetramera McKinn. One isolation from surface of Soil IV.

Hormodendron viride (Fres.) Sacc. Occasional in the soils studied. Other *Hormodendra* are found also in soil: *H. cladosporoides* (Fres.) Sacc. is commonest; this we refer to its form *Cladosporium herbarum.*

Hyalopus ater Cda. group. One isolation from a surface soil.

Monilia implicata Gilm. & Abbott. A *Monilia*, apparently this species, was obtained from the surface soil of a wheat field.

Paecilomyces varioti Bain. (*Penicillium divaricatum* Thom). This fungus, found rather commonly in butter in Manitoba (4), appeared in two isolations, one from B and one from C horizons.

Penicillium cyclopium Westl. Rare in surface soils.

Penicillium ?diercksii Biourge. One isolation from a forest soil.

Penicillium expansum (Link) Thom. This species, commonly reported from soils, was definitely identified only once from Manitoba soil, from the lower layer of a muskeg.

Penicillium flavi-dorsum Biourge. Also found in lower layer of peat.

Penicillium ?fuscum Sopp (as *Citromyces*). One culture from forest soil.

Penicillium guttulosum Gilm. & Abbott. This fungus was found to be common in the Forest Soils III and IV, particularly in sub-horizons A_2 to C.

Penicillium herquei Bain. & Sart. series. Found in meadow soil.

Penicillium nigricans Bain. group. A culture from forest soil.

Penicillium ?palitans Westl. From the lower layer of a muskeg.

Penicillium ?sublateritium Biourge. Also from the third layer of the peat.

Penicillium tardum Thom. From the B horizon of a forest soil.

Penicillium ?terlikowskii Zaleski. From the third layer of peat in a muskeg.

Penicillium thomi Zaleski (not *P. thomii* Maire). About 25 isolations from forest soil and peat. Close to *P. intricatum*, which is very common in Manitoba soils.

Periconia byssoides Pers. One isolation from meadow soil.

Periconia felina March. Thirty isolations from sub-horizons A_1, A_2 and B_1 of Meadow Soil I, Profiles 1 to 4.

Phoma hibernica Grimes, O'Conn. & Cumm. This fungus is common in butter in Manitoba (4). Three or four soil isolations produced pycnidia with pinkish spore masses and appeared identical with *P. hibernica* as it appears after some weeks in culture from butter. This fungus therefore may reach butter from the soil.

Sphaeronaema ?spinella Kalch. One isolation.

Sphaeropsis sp.? One isolation from the A_0 sub-horizon of a forest soil produced pycnidia with dark spores 14–17 × 6–8μ.

Sporotrichum pruinosum Gilm. & Abbott. Two isolations.

Stachybotrys sp. One isolation from the B horizon produced rough conidiophores and large rough dark spores 15–21 × 8–13μ. No description was found to fit this species.

Stemphylium macrosporoideum (Berk.) Sacc. One isolation, determined by Dr. Wiltshire. Eleven other isolations of *Stemphylium* could not be determined.

Tuberculariа ?vulgaris Tode. One isolation produced pink sporodochia and hyaline spores resembling those of *T. vulgaris*.

Verticillium ?nubilum Pethybr. A half-dozen isolations from surface soils produced verticillate conidiophores with spores 4–8 × 2–3μ. The cultures become dark with chlamydospores measuring 6–10 × 6–8μ. These cultures agree fairly well with the description by Pethybridge (13).

Volutella roseola Cke. One isolation from a surface soil agrees in detail with this fungus reported from soil by Bayliss Elliott (2).

Analysis of the Micro-organisms in a Profile

It seems unnecessary to give the complete protocols of fungi from the 12 profiles studied, since they are analyzed in subsequent sections. We give as an example, however, the identifications from Profile 8 of Soil III.

I. Fungi isolated by the standard technique, i.e., aerobically at 25° C., from which all colonies were picked from representative plates for identification (see 3, pp. 253–254).

Sub-horizon A_0: *Absidia orchidis*, 2 cultures; *Acrostalagmus albus* var. *varius*, 2; *Alternaria* sp., 2; *Aspergillus okazakii*, 1; *Coniothyrium* sp., 1; *Geomyces vulgaris*, 2; *Mortierella isabellina* var. *ramifica*, 4; *M. vinacea*, 5; *Mucor ?sylvaticus*, 2; *Penicillium braziliense*, 3; *P. ?canescens*, 1; *P. chrysogenum*, 2; *P. janthinellum*, 15; *P. lilacinum*, 2; *P. thomii*, 10; *P.* spp., 6; *Trichoderma album*, 3; *T. lignorum*, 4; *Verticillium ?glaucum*, 1; *V. terrestre*, 1.

Sub-horizon A_1: *Cephalosporium* sp., 1; *Penicillium rugulosum*, 1; no spores produced, 1.

Sub-horizon A_2: *Absidia spinosa*, 2; *Cephalosporium acremonium*, 2; *Cladosporium herbarum*, 1; *Cylindrocarpon heteronemum*, 5; *Mortierella vinacea*, 3; *Mucor racemosus*, 1; *Penicillium guttulosum*, 17; *P. janthinellum*, 2.; *P. restrictum*, 4; *P. restrictum* variety, 5; *P. thomii*, 1; *P.* spp., 7; *Phoma* sp., 1; *Trichoderma album*, 1; *T. koningi*, 4; *Verticillium* sp., 4; Unknown, 5.

Horizon B: *Cephalosporium* sp., 9; *Cylindrocarpon candidum* var. *majus*, 1; *C. heteronemum*, 1; *C. macrosporum*, 1; *Gliocladium roseum*, 2; *Mortierella vinacea*, 2; *Penicillium guttulosum*, 13; *P. intricatum*, 1; *P. restrictum*, 2; *P. restrictum* variety, 21; *P. thomii*, 3; *Trichoderma album*, 2; *T. glaucum*, 1; *T. koningi*, 1; Unknown, 3.

Horizon C: *Cladosporium herbarum*, 2; *Cylindrocarpon macrosporum*, 2; *Geotrichum candidum*, 2; *Mortierella elasson*, 5; *M. vinacea*, 1; *Penicillium guttulosum*, 12; *P. restrictum*, 1; *P. thomii*, 1; *Trichoderma album*, 1; *T. lignorum*, 4; Unknown 2.

II. Fungi isolated at 37° *C., i.e.*, the dilution plates were incubated at 37° and representative plates picked as before.

Sub-horizon A_0: *Aspergillus fumigatus*, 3; *A. nidulans*, 1; *A. niger*, 1; *Trichoderma koningi*, 5.

Sub-horizon A_1: *Trichoderma koningi*, 11.

Sub-horizon A_2: *Trichoderma koningi*, 2. Horizons B and C: no fungi obtained.

III. Fungi isolated anaerobically, i.e., the dilution plates were incubated in an atmosphere of CO_2.

Sub-horizon A_0: *Mucor* spp., 3; *Trichoderma album*, 3; *T. lignorum*, 5; no spores, 1.

Sub-horizon A_1: *Penicillium* sp., 3;

Sub-horizon A_2: *Mucor hiemalis*, 2; *Penicillium* sp., 4; *Trichoderma album*, 2; *T. lignorum*, 1; *Zygorhynchus moelleri*, 1.

Horizon B: *Cylindrocarpon candidum* var. *majus*, 4; *Trichoderma lignorum*, 1; *Verticillium ?glaucum*, 1.

Horizon C: *Colletotrichum* sp., 3; *Trichoderma koningi*, 1.

IV. Fungi isolated at about 6° *C., i.e.*, the dilution plates were incubated in a refrigerator.

Sub-horizon A_0: *Mucor ?sylvaticus*, 1.; *Penicillium janthinellum*, 7. A_1: no data.

Sub-horizon A_2: *Cladosporium herbarum*, 1; *Cylindrocarpon macrosporum*, 1; *Mortierella ?vinacea*, 2; *Mucor hiemalis*, 2; *M. ?sylvaticus*, 1; *M.* sp., 3; *Periconia felina*, 1; *Trichoderma album*, 1; *?Verticillium* sp., 3.

Horizon B: *Cylindrocarpon macrosporum*, 3; *Mortierella vinacea*, 5; *Penicillium lilacinum*, 1; Horizon C: *Cylindrocarpon macrosporum* 3; *Mortierella elasson, 1;* no spores, 1.

It may be mentioned that the data as to the numbers of fungi per gram of soil were obtained from the original isolation plates. The relative frequencies of different species of fungi were obtained by transferring all young colonies from representative plates to test tubes. These were incubated and subsequently the fungi were identified insofar as was possible.

The Important Soil Fungi of Manitoba

In an earlier paper (3) 121 different fungi were reported from Manitoba soil. In this paper 56 are added. Some of these 177 fungi are determined to genus only. The preceding paper (3) presented an analysis of the relative frequency of the species identified from 2565 colonies transferred "at random" from the dilution plates. In the work on soil profiles here reported 2163 colonies were studied similarly, so that information is available now as to the distribution among species of a "random sample" of 4728 Manitoba soil fungi. An analysis of the more important genera and species follows (see also Table I).

The "standard" isolation technique (23, see also 3) has the advantages of providing media upon which many kinds of fungi will grow, of making available comparable data on numbers of fungi per gram of soil and on the species present in various profiles or in various regions of the world. There are certain obvious disadvantages, however, to the routine methods of isolating fungi from the soil. These may be eliminated in part by plating from higher dilutions of soil to obtain species which grow more slowly, by incubating at high and low temperatures and under anaerobic conditions, and by the use of special media for certain fungi. All these methods have been utilized in Manitoba, although efforts have not yet been made to obtain species of *Pythium*, Saprolegniales, or Hymenomycetes from the soil.

Phycomycetes

Mortierella has proved to be the most abundant Phycomycete in the soil profiles examined. *Mortierella isabellina* var. *ramifica* was previously (3) found twice in surface layers of forest soil: now it has been identified 40 times, from forest and especially from peat, in both of which it occurs usually in the upper horizons. It was not found in Soil I. *M. elasson* was especially common in the C horizon of Meadow Soil I, and to a lesser extent in the B_2; it was found also 7 times in the C horizon of forest Soil III, but not in the A horizon of either soil, and not in peat. *M. vinacea* was common in forest soils and fairly common in peat. It was usually found in the upper sub-horizons. Thus the evidence suggests that species of *Mortierella* may be characteristic of certain types and horizons of soil.

Species of *Mucor* are fairly common in the A horizon of forest soil, rare in the B horizon, and were not isolated from any C horizon. They did not develop from Soil I. Specific determinations in the genus *Mucor* are difficult.

Absidia was found somewhat more commonly than *Mucor* in the profiles examined. *A. spinosa* was isolated 29 times, 22 of which were from Soil I. *A. orchidis* was obtained 15 times, 12 of which were from forest soil. *Absidia* like *Mucor*, is more common in the A horizon.

Rhizopus was isolated infrequently from the soil profiles studied, but was found rather commonly in surface soil in our previous work (3). *Zygorhynchus* is rare: it has been obtained only seven times from Manitoba soil. The few other Mucorales found may have been only "accidentally" present in the soil.

Plant pathologists in western Canada have demonstrated that species of *Pythium* occur in soil and cause injuries to crops. *Pythium* does not appear, however, in ordinary soil isolations. A special technique would be required to demonstrate the frequency of this important genus in the soil.

Ascomycetes

Isolations producing asci readily in culture were obtained from soil rarely, and those found usually were not true soil fungi. However, certain Sordariaceae such as *Chaetomium* spp. grow on debris and play a part in its disintegration in the soil. The larger Ascomycetes, such as Helvellales and Pezizales, often grow on decaying leaves or rich soil, but are rarely obtained in soil cultures. The points mentioned in the next paragraph apply also to many of these Discomycetes and to certain Pyrenomycetes.

Basidiomycetes

The isolation technique ordinarily used by the soil microbiologist yields little information on the flora of Hymenomycetes and Gasteromycetes known to be abundant in the soil, particularly in forests. Mycelium showing clamp connections was conspicuous by its absence in the cultures studied. Some of the sterile mycelia or certain cultures resembling *Cephalosporium* may have represented haploid stages of Basidiomycetes. A ramble through the woods or even over the prairie during a damp autumn in Manitoba gives one the impression that the large Basidiomycetes constitute the predominant fungus flora, and much mycelium can be traced from the bases of mushrooms, etc. into the leaf mold, the soil, or to twigs or roots. We have records of about 570 species of Agaricaceae in Manitoba, the large majority of which grow "on the ground". About 100 other Clavariaceae, Hydnaceae, Boletaceae, Lycoperdales, etc. also are to be found growing from the soil or fallen leaves; and about 200 other Polyporaceae, Thelephoraceae, etc., are to be found on wood. This large assemblage of fungi, giants as compared with the tiny (but abundantly sporulating) colonies of *Penicillium* or *Trichoderma* in the soil, should be taken into consideration in appraising the soil fungi. These Basidiomycetes play a very important part in the early stages, at least, of the disintegration of woody tissue and leaf mats, but the fungi isolated by plating soil are more truly "soil fungi" in that they commonly live upon organic matter sufficiently decomposed to be considered part of the soil itself.

Spores of rusts and smuts fall in numbers upon the soil, but only rarely does one obtain a culture which can be identified as one of the Ustilaginales.

Fungi Imperfecti

The great majority of fungi isolated from the soil must be placed in the Fungi Imperfecti, as one very rarely finds an ascus stage developed in the cultures kept in the laboratory.

Penicillium constituted nearly half the isolations from various horizons (920 cultures in a total of 2163), and was found in slightly over half the isolations previously (3) recorded (1404 cultures in a total of 2565). *Penicillium* is a very difficult genus. Accordingly we have not recorded species unless first determined by Thom. As the work progressed greater proficiency in identifying the commoner soil types by comparison with the determined cultures was attained, with the result that each lot sent to Dr. Thom tended to increase in difficulty and a score or so of forms were encountered which did not fit previously known species. It seems evident that some species of *Penicillium* are characteristic of certain types or horizons of soil: thus five species listed above were found only in the third layer of a muskeg; *P. guttulosum* was found to increase with depth in forest soil; *P. thomii* occurs throughout forest soil, rarely elsewhere; *P. terrestre*, so common in soils that had grown wheat (3, p. 264) was found only five times in the virgin soils herein analyzed; P. *chrysogenum* also appears to be commoner in cultivated soils; *P. intricatum* was found only occasionally in forest, but abundantly in most other soils; *P. lilacinum* was found especially commonly in Meadow-prairie Soil I; *P. rugulosum* was found fairly frequently in peat, where other species common in forest and field were mostly absent.

Aspergillus was not found to be common in the horizons studied, and when found was usually in the A horizon (49 isolations from A, and five in B and C together). Further evidence that *Aspergillus* prefers high temperatures is provided by the results of isolations made at 37° C. from which 27 of the 49 isolations developed. No *Aspergillus* developed in plates incubated in the refrigerator or anaerobically. *A. niger*, so common in southern soils, appeared only once in the course of the present study, from the A_0 of a forest soil incubated at 37° C. This corroborates our previous finding (3, p. 259) that *A. niger* is very rare in Manitoba. *A. fumigatus* was obtained ten times from dilutions incubated at 37° C. *A. flavipes* again was the commonest *Aspergillus*, appearing 25 times, 23 of which were from Soil I and 16 of these from the 37° C. isolations. *A. flavus* was found once in each horizon of Soil I. *Aspergillus* has been isolated only 104 times from Manitoba soil, whereas *Penicillium* has appeared 2324 times.

Trichoderma is much commoner in Manitoba soil than is *Aspergillus*, and is of particular interest because it is known to be "one of the most active groups of soil fungi decomposing celluloses, proteins, pectins and other complex organic substances" (23, p. 703) and *Trichoderma* has recently attracted considerable attention as a parasite of other fungi as was first recorded by Weindling (25) and independently found by the writers (3). Weindling (26–29) and others (8) have studied this parasitism further and have sought to prevent

certain plant diseases by adding *Trichoderma* to the soil. The recorded species of *Trichoderma* do not appear to be clear-cut (see 10), but we have identified species as follows from the profiles studied: *T. album*, 19 isolations; *T. glaucum*, 15; *T. koningi*, 91; *T. lignorum*, 95. *Trichoderma* is the commonest fungus on plates incubated anaerobically, and is rather commonly found on those incubated at 37° C., but only once did we get a *Trichoderma* (*T. album*) on plates held in the refrigerator. *Trichoderma* is especially common in forest soils throughout all horizons and is fairly common in peat. It was surprising, however, to find it only eight times from the four profiles of Soil I and not at all from the alkalinized Soil II. Weindling and Fawcett (29) found *Trichoderma* more active in acid soils.

Cylindrocarpon has proved to be a rather common fungus in most profiles studied, having appeared 99 times: in A horizon, 37, in B, 46, and in C, 16 isolations. Its greater prevalence in the present isolations than in those reported previously for Manitoba ((3) 24 isolations only) is due perhaps to its greater abundance in sub-surface horizons. *Cylindrocarpon* grows readily at low temperatures, being the commonest fungus on plates incubated in the refrigerator. It is rather common on plates incubated anaerobically, but was not obtained at 37° C. *C. macrosporum* was obtained 51 times; *C. candidum* 11, and its var. *majus* 21 times; *C. heteronemum* 14 and *C. didymum* 2. *Cylindrocarpon* was not obtained from or below peat.

Fusarium was found sparingly in the profiles of virgin soil studied (34 cultures, whereas the surface soils previously reported (3), most of which were under cultivation, yielded 128). *Fusaria* were commonest in Meadow-prairie Soil I (28 cultures); rare in forest soils (5 cultures), and only one culture was obtained from peat. Most of the cultures were from the A horizon, although they were obtained from B and C also. *F. oxysporum* and its varieties were the commonest forms isolated, but *F. coeruleum*, *F. poae*, *F. Solani* var. *martii*, *F. scirpi* var. *acuminatum*, *F. equiseti* form 1, and *F. sporotrichoides* were also found in the profiles. Several cultures of *Fusarium* were obtained from plates incubated anaerobically, but none at 37° C. Reinking and Manns (15, 16, 17) have recorded the *Fusaria* from several tropical soils, and have classified the various species isolated as "soil fungi" or "soil invaders." The lists of soil *Fusaria* from the tropics resemble the list from Manitoba soil; as Reinking (14) states, "many of the common *Fusaria* present on plants and in the soil of the temperate zone are distributed in the tropical regions." The species mentioned above from virgin Manitoba soil may be considered "soil *Fusaria*"; some of those previously (3) isolated from cultivated soils, such as *F. culmorum*, are apparently "soil invaders." Ziling (30) found *Fusaria* in Siberian soils very similar in numbers and kinds to those here reported.

Helminthosporium sativum causes serious root rots of cereals; it was isolated four times from the A horizon of Meadow-prairie Soil I, but from no other virgin soils. It is therefore a native of Manitoba grass land. Forty samples previously analyzed (3) of soil cropped for years with wheat showed about

the same sparse occurrence of *H. sativum*. It is probably prevented from accumulation in many cultivated soils by the activities of other soil microorganisms, such as *Trichoderma*. *Helminthosporium* seems to be of little importance in the soil, except for the potential parasitism of *H. sativum* on the Gramineae; but it may be that some or all species do not sporulate readily in the soil.

Rhizoctonia solani is known to vegetate in the soil as a sterile mycelium. It is therefore not likely to appear often on dilution plates from soil. Cultures with the mycelial and sclerotial characters of *R. solani* were obtained six times from virgin soil. This fungus, some strains of which are actively parasitic to higher plants, must therefore be fairly prevalent in such soils. It often causes injury to crop plants in Manitoba.

Cephalosporium has occurred as about 2% of all Manitoba soil fungi; 57 isolations attributed to this species were found in the profiles studied, 17 of which developed under anaerobic conditions but none at high or low temperatures (see Table I). This fungus appears rather evenly distributed through all soils and all horizons. *Cephalosporium* is an uncertain genus in that conidial or "oidial" stages of several fungi, including certain Hymenomycetes, may resemble the spore heads of *Cephalosporium*. Bayliss Elliott (2), for example, reports a conidial stage of *Volutella roseola* to agree with *C. acremonium*. *C. curtipes* was identified 11 times from the soil profiles, *C. humicola* 9, *C. acremonium* 5, undetermined species 32.

Verticillium occurs occasionally in soil (24 cultures, or 1% in the profiles). It is found especially in the A horizon of forest soil. The soil *Verticillia* need critical taxonomic study.

Alternaria is found occasionally in virgin soils (19 cultures from profiles) and this genus was noteworthy for its even, though sparse, distribution through all soils studied and in all horizons; it developed five times in anaerobic cultures and once each at 37° and at 6° C. Three cultures were identified as belonging to the *A. tenuis* group, the others remain unidentified. The related genus *Stemphylium* is somewhat less common (12 cultures) but was found occasionally in each type of soil.

Cladosporium herbarum was obtained only 16 times from the virgin soil profiles, whereas 69 cultures were obtained previously (3) from surface soils most of which were cultivated. Four cultures of the 16 developed at 6° and none at 37° C. or anaerobically. *Cladosporium* was found in both meadow and forest soils, from A to C horizons.

Phoma and *Coniothyrium* (32 and 22 isolations respectively) are rather common throughout both meadow and forest soils. These are interpreted to arise from spores from pycnidia of various fungi growing on decaying roots and other plant parts.

Monotospora daleae Mason (11) deserves mention because it was isolated 15 times from the A and B horizons of Meadow Soil I and not from any other

soil. It was previously (3, where it is recorded as *Mycogone nigra*) found 18 times, principally from a wheat field. These figures suggest that *M. daleae* develops in soil containing parts of Gramineae.

Metarrhizium sp., previously (3) reported as common in soil of wheat fields, was conspicuous by its absence, save for one doubtful isolation, from the virgin soils studied. Dr. J. E. Machacek finds the same *Metarrhizium* occasionally in diseased cereal roots.

Periconia felina is noteworthy because it appeared 31 times from the A and B horizons of Meadow Soil I, but has not been obtained elsewhere in Manitoba.

Gliocladium occurs throughout meadow and forest soil and can be considered a true "soil fungus." *Geomyces vulgaris* perhaps is also a widespread inhabitant of soils. *Acrostalagmus* spp. and *Hymenula affinis* are occasionally obtained from various soils in Manitoba.

Other Fungi Imperfecti isolated from the soil, such as species of *Botrytis*, *Geotrichum*, *Monilia*, *Scopulariopsis*, *Sporotrichum*, *Haplographium* and *Colletotrichum*, have been obtained so infrequently in Manitoba that little can be said about their possible roles in the soil. A number of other fungi, such as species of *Cytospora*, *Dactylium*, *Tubercularia*, and *Hyalopus*, evidently appear in cultures from the relatively few spores that have fallen upon the soil from a saprophytic or parasitic growth upon the plants above, or even within, the soil. When it is remembered that an average surface soil in Manitoba contains about 125,000 viable spores (including occasionally bits of mycelium) *per gram*, it becomes clear that the extraneous spores which fall upon the soil must make up but a minute fraction of the population of fungi present.

Undetermined fungi inevitably remain as a residue from large numbers of isolations from soil. Nearly half of the 170 transfers which were not determined at the time of writing the previous paper (3, p. 267) have now been determined, but, for lack of spores or other reasons, no identification was made of 168 of the 2163 fungi isolated from soil profiles.

Table I summarizes all species which were isolated 12 or more times (*i.e.*, 0.5% or more) from the soil profiles studied. When the genus name alone is given, the entries refer to all cases in which the total isolations were 12 or more, but not that number under any one species of the genus. Table I also includes the number of isolations, of the species of genera included, obtained from transfers from plates incubated at 37° C., at 6° C., or anaerobically.

Fungi in Different Soil Horizons

The surface sub-horizon, A_0 or A_1 as the case may be, receives many spores from the air, so that fungi extraneous to the soil are most likely to be obtained there, but in small numbers. In Table I most or all such fungi have been eliminated. *Aspergilli* and certain *Penicillia* and other fungi show a tendency to decrease with depth. On the other hand *Mortierella elasson*, *Cylindrocarpon*

TABLE I

NUMBER OF ISOLATIONS OF COMMONER FUNGI FROM SOIL HORIZONS

Fungus	Soil I					Soil III				
	A_1	A_3	B_1	B_2	C	A_3	A_1	A_2	B	C
Absidia orchidis				2	1	4				
Absidia spinosa	12	5	2	3		5		2		
Mortierella elasson		1†	2	19	30 7† 2§					6 1†
M. isabellina ramifica						6	6			
M. vinacea	1*					7	6	3	3	1
Mucor spp.	2 2§	2	1* 1†			3		1§	1§	2
Alternaria spp.	2 2§	2	1* 1†			3		1§	1§	2
Aspergillus flavipes	3 7*	4 9*								
A. spp. all others	2 5*	1	1	1	2	4 5*	2			1
Cephalosporium spp.	1	2	1 7§	1			2	9	13	1
Cladosporium herbarum	4 3†						1	5 1†		2
Coniothyrium spp.		2 2§	4			3				1
Cylindrocarpon candidum var. *majus*	1	1 1§	5 1§	4	3				1 4§	
C. macrosporum	2 4†	1	6 4†	10 2§	3		1 1†	1 1§ 3†	4	4 3†
C., other species	4	5	5 1§ 2	2			5	1		
Fusarium spp.	18 7§ 1	2				1§			1§	
Gliocladium spp.		8	7			1			2	
Monotospora daleae	1 1†	6	6	1						
Penicillium gutulosum					1		1	17	13	12
P. intricatum	28 2†	61 6†	2 2†	2 1†				1	8	
P. janthinellum	38	52 7†	21			23		2	1	
P. lilacinum	17 1§	6	5	2		2			1†	
P. purpurogenum	1					7		1*	6 5*	
P restrictum var.								6	21	1
P. rugulosum						1				1
P. thomi							1	1	3	
P. thomii				1		19	2	1	15	18
P. spp., all others	4 1§	7 1†	8	6 2†	4	17 1*	8	8	11	6

*Developed at 37° C. †Developed at 6° C. §Developed anaerobically.

TABLE I—*Continued*

NUMBER OF ISOLATIONS OF COMMONER FUNGI FROM SOIL HORIZONS—*Continued*

Fungus	Soil I					Soil III				
	A_1	A_2	B_1	B_2	C	A_0	A_1	A_2	B	C
Periconia felina	2 1†	18 2§	7							
Phoma spp.	12	5	1	1		7	1	1		
Trichoderma album			1†			4 3§		3 2§	3 1§	1
T. glaucum								2	1	
T. koningi	1§					5 7* 3§	10 1* 1§	6 2* 7§	3 2§	3 3§
T. lignorum	1§	2	3			8 1* 12§	5		12 2§	9
Verticillium spp.			1			5		5	1§	1§

Fungus	Soil IV					Soil V		
	A_0	A_1	A_2	B	C	1	2	3
Absidia orchidis	5 1§			2				
Absidia spinosa								
Mortierella elasson	2?				1§			
M. isabellina ramifica	3			1		10	11	3
M. vinacea	8	10	4	2		7	3	3
Mucor spp.		1	1 1*				1	
Alternaria spp.		1	1 1*				1	
Aspergillus flavipes		2						
A. spp. all others		2	2			1		
Cephalosporium spp.	6	1	6§	2	4§		1	
Cladosporium herbarum								
Coniothyrium spp.	9 1*							
Cylindrocarpon candidum var. *majus*								

**Developed at 37° C.* †*Developed at 6° C.* §*Developed anaerobically.*

TABLE I—*Concluded*

NUMBER OF ISOLATIONS OF COMMONER FUNGI FROM SOIL HORIZONS—*Concladed*

Fungus	Soil IV					Soil V		
	A_0	A_1	A_2	B	C	1	2	3
C. macrosporum								
C., other species					1 1§			
Fusarium spp.				1 1§	1§			1
Gliocladium spp.	2§		1§	1§				
Monotospora daleae								
Penicillium gultulosum	2	10	21	23 2§	23 14§			
P. intricatum	5							
P. janthinellum	21	4	1		5			
P. lilacinum								
P. purpurogenum								
P. restrictum, var.		1		5				
P. rugulosum						9	7	7
P. thomi				12	5			14?
P. thomii	19	7		2		19*		1
P. spp., all others	9 1§	7 3§	5 5§	4 1§	1 1§	34 5§	14 4§	44 4§
Periconia felina								
Phoma spp.	3 1§							
Trichoderma album				1§				
T. glaucum	1§	1 1§		2§		2§	2 3§	
T. koningi	6 1* 2§	2 1§	1 5* 2§	1 2§	2§	3 3* 1§	2	1
T. lignorum	1 8§	4 7§	7 3§	2 2§	1§		4 1§	
Verticillium spp.		8	2	2				

*Developed at 37° C. .Developed at 6° C. §Developed anaerobically.

macrosporum, and *Penicillium guttulosum* showed distinct increases in deeper soil horizons. As might be expected, however, there is usually no striking difference in the fungus-flora of different horizons.

Horizon C begins at 17 to 27 inches in Profiles 1 to 10. In Profiles 11 and 12, the peat bog, the third layer (not a horizon) began at 58 and 70 inches. *Aspergilli* were not common in C or B horizons, and the *Penicillia* were rare in the C horizon of Meadow Soil I. The only *Penicillium* common in C horizons of Forest Soils III and IV was *P. guttulosum*. Several species of *Penicillium*, however, made up the majority of the organisms in the lower layer of peat. Anaerobic and low temperature fungi are more common in the C and B horizons than in the A horizon. It is possible that strains of fungi differing physiologically and even morphologically may develop during a long sojourn deep in the soil. We were at first inclined to think that fungi from lower horizons more frequently failed to produce spores than those from upper horizons, but such is not really the case, although spore production may be delayed. Only 16 isolations from C horizons could not be identified. Some of these produced spores.

The Fungi in Peat

One striking feature of the peat studied was the marked decrease of fungi with depth. Waksman and Purvis (23) found the same phenomenon, no fungi being obtained in peat from Maine at 30 inches and below. *Mortierellae* were common in peat in Manitoba (see Table I). *Penicillia* constituted 60% of all fungi isolated, but were for the most part species not found in other soils studied. Peat is, of course, very different from ordinary soil and has a different flora of fungi.

Anaerobic Fungi in the Soil

Lower horizons of heavy soils must often be deficient in free oxygen. Yeasts and certain other fungi are known to be capable of living anaerobically, or at least in media with low oxygen content. We have not seen, however, reports on the anaerobic fungi of the soil.

TABLE II

NUMBERS AND PERCENTAGE OF FACULTATIVELY ANAEROBIC FUNGI PER GM, AS COMPARED WITH TOTAL FUNGI

Profile	Horizon	Total fungi per gm.	Anaerobic fungi	
			Number per gm.	Percentage
6	A_0	117,000	1120	0.96
	A_1	15,625	1010	6.5
	A_2	4,250	590	13.9
	B	995	540	54.3
	C	120	50	41.7
9	A_0	205,000	1326	0.65
	A_1	65,000	970	1.5
	A_2	7,500	1650	22.0
	B	14,750	2380	16.2
	C	1,840	680	37.0

Anaerobic conditions were obtained by introducing carbon dioxide into a chamber until the air was displaced. Soil dilution plates were incubated at the bottom of the chamber, closed except for two tubes, through one of which carbon dioxide constantly entered slowly, the other serving as exit

tube. The figures given by Timonin in the previous paper (20) show that from 20 to 2850 anaerobic fungi were found per gram in Soils I, III and IV, and up to 35,000 per gram in peat. In the peat about 10 to 20% of the total fungi in all three layers are facultatively anaerobic, but in the other soils there is a noteworthy percentage increase of anaerobes with depth. This is brought out in Table II.

As can be seen in Table I, relatively few *Penicillia* capable of developing anaerobically were found. Thom (19, p. 84) states that "Absence of free oxygen practically stops the growth of *Penicillium*." A few soil forms, however, can make good growth in an atmosphere of carbon dioxide. No *Aspergilli* were obtained anaerobically, but *Trichoderma* appeared commonly. Species of *Cylindrocarpon*, *Fusarium*, and Mucorales were the other fungi principally found. *Verticillium*, *Alternaria*, and *?Colletotrichum* were also obtained.

All fungi which developed in the atmosphere of carbon dioxide and which were transferred to test tubes for study, were found to grow readily aerobically. The faculty of growing anaerobically must aid soil fungi materially in deeper soils and in peat bogs.

Soil Fungi Which Develop at 37° C.

Soil dilutions from several horizons were incubated at 37° C. These plates showed a striking prevalence of *Aspergilli*. *Trichoderma koningi* also can flourish at a high soil temperature. Relatively few *Penicillia* developed at 37° C. (see Table 1). Certain fungi not usually found in soil cultures appeared, such as *Humarina*, *Xylaria*, and *Haplographium*. Most of the high temperature fungi were found at or near the surface of the soil which of course is subjected to the highest summer temperatures.

Soil Fungi Which Develop at Low Temperatures

Dilution plates incubated in a refrigerator at about 6° C. developed *Cylindrocarpon*, Mucorales, certain *Penicillia*, less frequently *Cladosporium*, and rarely other fungi. No *Aspergilli* and only one *Trichoderma* was obtained (see Table I). The low temperature fungi show an increase, relative to total fungi, with greater depth of soil. It is to be noted from the tables in the preceding paper (20) that low temperatures commonly prevail in the lower horizons of soil in Manitoba: for example, 9° C. in the C horizon below peat on July 19 and 11° C. in B and C horizon of Profiles 9 and 10 on the same date. On May 15 the C horizon was still 0° C. in Profile 2.

Conclusion

Some years ago the question arose as to the occurrence of a definite fungus-flora of the soil. The answer seems clearly in the affirmative. Indeed, Waksman (22) by 1916 was able to postulate a "typical soil flora" of fungi which has in general been verified by subsequent work in various parts of the world. Jensen (10) records a list of fungi from soils in Denmark which

is very similar to the list from Manitoba; both show the relative infrequency of *Aspergilli* in northern areas. Ziling (30) finds a flora of soil fungi in Siberia very like that in Manitoba and elsewhere. Miss Nobles (12) isolated 24 genera of fungi from the soil of southern Ontario, all but one or two of which have been found in Manitoba, and in about the same relative abundance. Many soil fungi are very widely distributed, as mentioned above for *Fusaria*. Chaudhuri and Sachar (5) report about 30 species of fungi from Punjab, India: about half of these have been found in Manitoba. In India *Aspergilli* are no doubt common soil fungi: one-third of the species recorded by Chaudhuri and Sachar belong to this genus. Weindling (27) makes the interesting suggestion that one reason for the prevalence of certain fungi in the soil is their ability to overcome their competitors and even to use their hyphae as food.

Despite the facts that many soil fungi are very widely distributed and the flora of any soil constitutes a very complex and fluctuating population, there is evidence that certain species of fungi may be characteristic of certain types or horizons of soil. This paper attempts to evaulate the significance of various species by considering their relative abundance in different horizons under high, intermediate or low temperatures of culturing, presence or absence of oxygen, and such other data as are available.

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THE EUROPEAN RED MITE, *PARATETRANYCHUS PILOSUS* C. & F. IN NOVA SCOTIA[1]

BY F. C. GILLIATT[2]

Abstract

Terminology, food plants, dispersion and economic importance of the European red mite, *Paratetranychus pilosus* C. & F., which affects deciduous fruit trees in America, are discussed. The mite, first observed in Nova Scotia in 1913, became of economic importance in 1925. The life history, obtained from an insectary and field study extending over a period of three years from 1930 to 1932, is recorded in detail. There are two complete generations and three partial generations in Nova Scotia. Fluctuations in temperature markedly affect mite development. Natural control of the winter eggs is important.

The European red mite, *Paratetranychus pilosus* C. & F., is a European fruit pest which was described in 1878 from Italy. It was first reported in North America by Ewing (6) in 1911, from the State of Oregon, and by Caesar (3) in 1912, from the province of Ontario. In view of the fact that it was present in such widely separated regions, it must have been introduced from Europe considerably earlier than 1911. During the following few years many new infested localities were found and at the present time the species is a pest of more or less importance in all, or at least most, of the deciduous fruit growing regions of North America.

In Nova Scotia, recollection of the presence of this mite by some entomologists can be cited as early as 1913, although at that time the species was not definitely known. In 1922, a number of orchards near the centre of the Annapolis valley were observed to be moderately infested. It was not, however, until 1925 that some special treatment to control this pest became necessary, and since then it has become increasingly imperative. At the present time it appears that the European red mite must be classed in the Annapolis valley as a permanent orchard pest which will doubtless fluctuate in numbers from year to year. Recognition of this led to a biological study of it being undertaken in Nova Scotia, during 1930, 1931, and 1932.

Terminology

It is evident from the available literature that this particular mite was described in 1878 by Canestrini and Fanzago (4) from Italy and named *Tetranychus pilosus*. In 1910, Zacher (15) erected the genus *Paratetranychus*

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in which he placed *pilosus*. Banks (2), in 1917, attempted to replace the genus of Zacher with *Oligonychus* of Berlese. This replacement was disputed, and an excerpt by McGregor and Newcomer (10) from Berlese, sets forth certain anatomical characters in disproof. In England, however, the species is known as *Oligonychus ulmi* and evidently workers there consider the genus of Berlese as being more applicable. Massie (8) mentions *Oligonychus ulmi* in a recent work, stating that it is known in the United States as *Paratetranychus pilosus*.

In America, the species was first confused with the better known *Tetranychus mytilaspidis* which attacks citrus fruits. As a result, for a number of years after its appearance in 1911, it was reported as *T. mytilaspidis* by many workers, (5, 6, 12) from different parts of the United States as well as from Canada (3). McGregor (9, 10) has evidently proved conclusively that the form attacking citrus trees and that infesting deciduous fruit trees are two distinct though closely allied species. The controversy appears to have ended, at least for the time being, and the former is known as *Paratetranychus citri* McG., and the latter *Paratetranychus pilosus* C. & F.

There are also a number of common names by which this mite is known. It is only natural, considering its introduction from Europe, that it is known in America chiefly as the European red mite. It has, however, been referred to in this country as the imported red spider, and Ross (13) of Ontario mentions the plum spider mite. In England, Massie (8) refers to this mite as the fruit tree red mite. It was called the fruit tree spinning mite by Trägårdh of Sweden, while in Italy it is known as the rose mite.

Food Plants

The European red mite infests all, or at least most, of the deciduous fruits. In Ontario, Ross (13) reports that the European plum is the most important host, with the apple next. Garman (7), from Connecticut, states: "Most damage is done to apple and plum." Newcomer and Yothers (11) list plum, quince, apple, and pear as the favorite food plants. In England, Massie (8) refers to this species as probably equally important on apple and plum. In Nova Scotia, apple is the outstanding host of this mite. The writer has observed a few adult mites as well as winter eggs on plum and pear, but the numbers were few and the trees always in proximity to infested apple orchards. The fact that the plum is not seriously attacked in Nova Scotia may be explained by the comparatively small number of orchards of this fruit, or it may be that the varieties grown are those least susceptible to attack.

Host plants other than the above which have been infested include peach, walnut, cherry, almond, grape, raspberry, hawthorn, mountain ash, elm, cedar, rose and chestnut. In no instance, however, has the writer been able to find records indicating that any of these plants have been seriously infested.

Economic Importance

In the Annapolis valley, the European red mite is to be found in all districts. It is chiefly in the central and more easterly sections, however, that the worst outbreaks have occurred.

Both the adult mites and immature forms feed by withdrawing the plant juices and chlorophyll from the foliage. The leaves lose their dark green color, become pale and assume a bronze color with an unhealthy appearance. When a considerable area is infested such orchards can be detected for some distance as a result of the pale color of the foliage.

It has been stated by Garman (7) that 12 to 33 mites per leaf cause browning of apple foliage and that 55 to 133 per leaf will cause severe browning. In Nova Scotia, from 125 to 140 female adults and 90 to 226 eggs and immature forms were counted per leaf on a considerable portion of the foliage where leaf injury was severe.

As a rule the mite population is not sufficient to cause noticeable foliage injury until nearly midsummer. At this advanced date the regular spraying for other pests has been completed and it is difficult to apply effective sprays for mites so late in the season. In consequence the pest is allowed, as a rule, to proceed unchecked as far as artificial control is concerned, until the following dormant season. It is difficult to estimate the total loss as a result of severe European red mite infestations, as much depends upon the original vigor of the trees, and the treatment following the outbreaks. In orchards where the foliage has been badly depleted of chlorophyll, the fruit is late in maturing, green and small. In the autumn, green, undersized fruit is particularly noticeable on heavily set trees, which otherwise would have produced a normal crop. The foliage of infested orchards drops prematurely, resulting in depleted vigor the following year. In such orchards there is a light bloom or frequently an entire lack of blossoms.

Dispersal

In the writer's opinion there is no large scale spread of the European red mite for any distance. Once a start is made, the increase takes place, under favorable conditions, largely within the orchard itself. There are, however, ample opportunities for spread over short distances in individual orchards or to adjoining ones. Traps consisting of glass slides 3 × 5 in., smeared with Nujol oil, were exposed on stakes in such a manner that they were always facing the wind. These were placed on the east side of infested orchards so as to receive the drift of the prevailing winds. The slides were set on August 1 and left exposed for the remainder of the season. At Tupperville, N.S., three such slides were exposed 25 ft. from infested trees. Only one adult female was caught. At Lakeville, N.S., the same number of slides were exposed and placed 100 ft. from the trees and no mites were captured. Considering the small size of the exposed surface the capture of even one mite is suggestive of a wind drift of mites. In the entire investigation, however, there has been no suggestion of a wind drift of mites on a large scale.

It seems that possibly more mites are transported by light or moderate winds than by gales. A severe windstorm was experienced in the Annapolis valley on August 6 and 7, 1930, and it was observed during the storm that the mites clung very tenaciously to the foliage. On August 8, the numbers of adult female mites were counted both on the windward and the leeward sides of the trees. The average numbers of mites were 297 per 100 leaves on the windward side and 273 on the leeward side. Had there been a wind drift of any importance a concentration of mites on the leeward side would probably have followed. Both at Annapolis Royal and at Berwick the mites appeared as numerous on the foliage after the storm as before.

After a storm in September 1930, bits of webbing which had been floating in the air were found caught by apple limbs. The webbing had been spun about the massed winter eggs on the limbs at the time of egg deposition. Owing to the storm some of this webbing had been dislodged, and adhering to it were winter eggs in appreciable numbers. It would appear that this may be an important source of distribution, or at least sufficient to make a start for new infestations at some distance.

In 1930, a fruit grower at South Berwick, N.S., stated that when picking Duchess apples he noticed adult female mites in considerable numbers on a white duster suit he was wearing. If mites are brushed off the trees in this manner, it is obvious that all operations in the orchard, such as spraying, cultivating, gathering fruit, etc., are responsible for the spread about the farm or to other points where operators may be called.

The most fruitful source of spread for long distances is of course the movement of nursery stock during the dormant season. In this way the winter eggs are shipped from one country to another.

Effects of Weather on European Red Mite

All stages of the mite are susceptible to modifications by variations in weather conditions. A few warm days in May bring on hatching of the winter eggs, but a sudden lowering of temperature, or a wet cold period after hatching has begun, almost entirely suspends emergence. Further, the young mites remain in a semi-dormant condition with apparently no development throughout a cold wet period. Often they were completely submerged in drops of water on the leaves for several hours in complete inactivity. This caused no apparent ill effects, as rising temperatures with drying of the foliage soon brought renewed activity. The life of the adult is likewise prolonged and fewer eggs are deposited by the females of the first and fifth generations than are deposited by midsummer generations when higher temperature prevails. Even a cool, dull period in the warmer and more settled portion of the summer will cause at least some slight prolongation of the various stages.

It is quite evident, therefore, that there is a close correlation between the prevailing average mean temperature and the development of the immature forms. This is shown in Table I, where the average mean temperature for

the period of immature development has been computed for each generation beginning with the first mite to hatch and extending until the last deutonymph moulted and emerged as an adult. In Table II is shown the relation between the average mean temperature and the incubation period.

TABLE I

AVERAGE LENGTH OF IMMATURE PERIOD OF EUROPEAN RED MITE IN RELATION TO PREVAILING AVERAGE MEAN TEMPERATURE, ANNAPOLIS ROYAL, N.S.

Year	Average length immature period. First generation. Days	Average mean temperature. First generation. °F.	Average length immature period. Second generation. Days	Average mean temperature. Second generation. °F.	Average length immature period. Third generation. Days	Average mean temperature. Third generation. °F.	Average length immature period. Fourth generation. Days	Average mean temperature. Fourth generation. °F.	Average length immature period. Fifth generation. Days	Average mean temperature. Fifth generation. °F.
1930	(59) 17.62	53.4	(61) 9.20	66.9	(67) 11.1	66.3	(80) 11.49	63.7	(32) 15.07	59.8
1931	(42) 13.2	58.2	(60) 11.75	63.7	(54) 10.1	68.1	(57) 11.96	64.4	(49) 16.9	57.6

Figures in brackets represent the number of individuals under observation.

TABLE II

AVERAGE LENGTH OF THE INCUBATION PERIOD OF THE EUROPEAN RED MITE IN RELATION TO PREVAILING AVERAGE MEAN TEMPERATURE, ANNAPOLIS ROYAL, N.S.

Year	Average length incubation. Second generation. Days	Average mean temperature. Second generation. °F.	Average length incubation. Third generation. Days	Average mean temperature. Third generation. °F.	Average length incubation. Fourth generation. Days	Average mean temperature. Fourth generation. °F.	Average length incubation. Fifth generation. Days	Average mean temperature. Fifth generation °F.
1930	9.21	67.0	9.53	67.1	9.81	65.6	11.49	63.8
1931	13.1	60.4	8.40	67.6	9.47	66.2	13.03	60.1

Life History

The life history of the European red mite includes the following stages: egg, larva, nymph and adult. Upon the hatching of the egg there emerges a six-legged mite, the larva. After the first moult the eight-legged protonymph appears. This is followed by the deutonymph after the second moult, and upon the moulting of the deutonymph the adult emerges. During the first half of the growing season, the so-called summer eggs, which hatch during the current season, are deposited on the leaves. Later in the season, beginning about midsummer, winter eggs are deposited on the limbs. This is the stage in which the winter is passed.

In Nova Scotia, there are five generations of the European red mite, two complete and three partial ones. The females of the first and second generations deposit summer eggs only. The third deposits a small percentage of winter eggs, and the fourth deposits summer and winter eggs in the approximate ratio 60 : 40. The fifth generation deposits winter eggs only.

Method of Study

The mites were reared individually in rearing cells, according to the methods described and used by Newcomer and Yothers (11). The cells were made of white felt $\frac{1}{4}$ in. thick, cut into squares $1\frac{1}{2}$ in. with a hole $\frac{1}{2}$ in. in diameter punched in the centre. Squares of thin celluloid were also cut to the same size and punched in a similar manner. One of these was glued to each square of felt, a small piece of paper bearing a number for records being inserted first. A similar square of celluloid, but unpunched, was also used with each cell. These cells were attached to individual leaves on a small apple tree growing near the laboratory. The cell was placed on the under surface of the leaf, with the felt next to the leaf. The unpunched square of celluloid was then put on the upper surface and the whole fastened to the leaf with paper clips. In this way a small circle of leaf was left exposed in the felt cell. To avoid escape of the mites a thin smearing of "tanglefoot" was put on the felt around the cell. To prevent the cells being blown from the trees a cotton screen was built on three sides and about one foot from the ground to provide a free circulation of air. One mite was transferred to each cell immediately upon emerging from the egg and reared to the adult stage.

The cells were examined daily, each change of development being recorded as it appeared. In order to obtain sufficient magnification it was necessary to use a binocular microscope with stand removed, and this necessarily made the work slow and tedious. Approximately 100 cells were used at each brood and it was possible to rear from 50 to 75 mites by this method. Newcomer and Yothers used the cells both for rearing the young mites and for mating the adults to obtain laying records, but in the writer's experiments, the cells proved to be entirely unsatisfactory for the latter purpose. As a rule the adults mated within the cells, and frequently the female oviposited two to four eggs, but then became restless and either escaped or became entangled in the sticky material. A rearing cell about one inch in diameter was substituted, but this proved little better than the smaller cell. A whole leaf was then given to each mated female, with a circle of tanglefoot at the base of the petiole to prevent escape. The majority of the females remained upon the leaf and apparently deposited their full complement of eggs. This method was, on the whole, quite satisfactory.

A diagram was made of both sides of the leaf with the principal veins sketched in, and the oviposition dates were obtained by daily observations, each egg as it was deposited being recorded and properly located on the diagram. From these records the hatching dates and thus the length of the incubation period were obtained.

On account of the possible early deposition of winter eggs, beginning with the third generation, instead of the females being restricted to the leaf, each was given the range of a short piece of spur. The tanglefoot, therefore, was put down from the leaf about two inches instead of around the leaf petiole. This appeared to parallel natural conditions for it gave the female an opportunity to deposit both summer and winter eggs. It should also be stated that all leaves in the cluster except the one on which the adult was placed, were previously removed and the short piece of spur gone over with a dry tooth brush to remove any possible old winter eggs or empty shells.

It has been stated by Newcomer and Yothers (11) that the individual females in the later generations lay either all summer eggs or all winter eggs, this conclusion being based on the fact that the eggs either all hatched or all failed to hatch during the current season. However, they apparently used the leaf cells throughout the season and the females had no opportunity of depositing winter eggs naturally upon the twigs. In any event, the writer's investigations in Nova Scotia did not support this hypothesis. Three females of the third generation which laid one winter egg each also deposited a number of summer eggs. In the fourth generation, 21 females out of a total of 35 laid both summer and winter eggs. On this basis 60% of the females of this generation laid both types of eggs.

In conjunction with the rearing work at the laboratory, frequent visits were made to various infested orchards scattered over the western portion of the Annapolis valley. This served as a check on the development of mites in the field in comparison with the rearing work.

Number of Generations

It has been stated by Newcomer and Yothers (11) that there are six complete generations and a partial seventh and eighth generation in Washington state; Garman (7) in the east suggests six generations in Connecticut, while Ross (13) considers there are at least six generations in Ontario. In Europe, Massie (8) states: "The number of generations in one season varies, but it is considered that there are at least four in an average season." Trägårdh (14) reports that there are four generations in Sweden.

The results obtained from the study of the European red mite during 1930 and 1931 indicate that there are five generations each year in Nova Scotia—two complete and three partial. The adults of the first and second generations lay summer eggs. The females of the third generation deposit a small percentage of winter eggs while the remainder and much larger number are laid on the foliage as summer eggs. In the rearing cages only three eggs were deposited on the twigs as winter eggs, this being a very small proportion of the total deposited. From orchard observations it was evident that a much larger proportion was deposited as winter eggs by the females of the third generation. On July 31, 1930, when oviposition by the females of the latter generation was practically complete, counts were made, in an orchard, of

the number of eggs on the leaves and on the twigs. These counts indicate that of a total of 3399 eggs, 3030 were on the leaves and 369 on the twigs, or 10.86% winter eggs. These results, though not strictly accurate owing to unavoidable factors in making the counts, are at least a fair representation of the proportion of winter eggs deposited by the females of the third generation. Eggs were also deposited by females of the same generation upon the fruit, principally about the calyx. These presumably were winter eggs as none hatched during the current season.

The adults of the fourth generation also deposited both summer and winter eggs. In this instance it was necessary to rely upon those deposited in the rearing cages to obtain the proportion of each, it being impossible to make accurate counts in the orchard. The percentages of winter and summer eggs deposited by the females of the fourth generation in 1930 were 45.2 and 54.8 respectively. In 1931, the percentages were 34.8 and 65.2 The eggs of the fifth generation were all deposited on the twigs as winter eggs. From the results of the rearing work over a two-year period it is evident that the eggs found on the limbs during the winter are an accumulation of those deposited by the females of the third, fourth, and fifth generations.

Overlapping of Generations

In Fig. 1, there is represented the seasonal history of the European red mite at Annapolis Royal during the years 1930 and 1931. The dark lines indicate the duration of the various generations, at the extreme left the date when the first larva emerged and at the right, when the last adult died. The

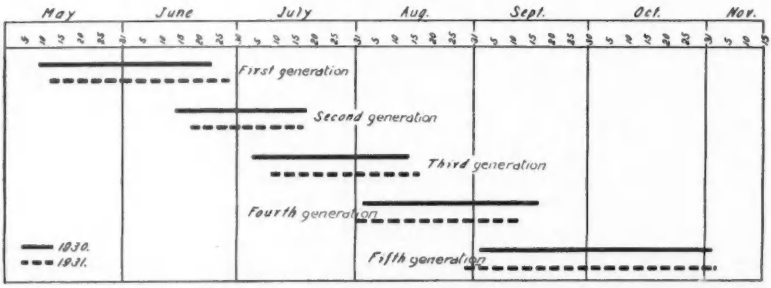

FIG. 1. *Seasonal history of the European red mite at Annapolis Royal, N.S., 1930 and 1931.*

extremes are necessarily represented in this chart; therefore, the actual number of mites overlapping is comparatively small. At no time during the season was there more than an overlapping of the latest part of one generation with the beginning of the following one. All field observations made in various parts of the Annapolis valley throughout the seasons of 1930 and 1931 indicated that there was no pronounced overlapping of the various generations. With a pest of this nature this is somewhat contrary not only to the general expectation, but also to the prevailing opinion.

Winter Eggs

The winter eggs are deposited upon the small outer branches and also on the larger limbs when these are free of loose scaly bark. They are largely laid on the under, or more protected, surface where they become massed in immense numbers and produce a red appearance which is distinguishable for some distance. A small proportion are also deposited upon the surface of the fruit more particularly about the calyx.

The eggs are bright red, maintaining their color throughout the winter without appreciable change until shortly before hatching when they turn somewhat paler. After hatching, the empty shells remain upon the limbs for some time and are silvery white and rather transparent. In form the egg is spherical, flattened at the poles, with fine ridges radiating from the visible pole. A short fine stalk projecting from this pole is visible only with a strong lens. In diameter the eggs vary from .152 to .162 mm. measured across the pole.

In 1930, the first winter egg was observed in the rearing cage on July 26, and in 1931, on July 28. In the orchard, winter eggs were laid at a slightly earlier date, the first being observed on July 22 in both years.

Under control, the last egg in 1930 was laid on October 27, and in 1931 on October 30. It is evident, therefore, that the winter eggs are deposited over a period of more than three months. It must be fully realized, however, that both of these seasons were exceptional since no frosts occurred until after the middle of October, some three to four weeks later than normal.

Summer Eggs

The summer eggs, as a rule, vary from a bright to a pale red. Some, however, are quite transparent with very little color. As the embryo develops, the eggs turn decidedly lighter in color and shortly before hatching have a rather watery, transparent appearance. In size they are slightly smaller than the winter eggs, varying from .145 to .160 mm. in diameter. In other respects the summer and winter eggs are similar.

The summer eggs are deposited on both surfaces of the leaves, with a somewhat larger portion on the under surface. A count of the eggs of the first generation on 86 leaves indicated 1505 eggs on the under surface and 1139 on the upper, or 57% and 43%. The larger number are laid along or near the midribs or larger veins.

The average incubation period of the eggs of the second, third and fourth generations in 1930 was between nine and ten days. Those of the fifth generation were about two days longer in hatching. In 1931, the eggs of the second and fifth generations hatched in approximately 13 days and those of the third and fourth in about nine days. In all generations there was some mortality of the summer eggs. This mortality, however, was too small to cause any noticeable suppression of the pest. The eggs which failed to hatch did not exceed 16% in any instance in the two years of investigation, and in the case of two of the 1930 generations were less than 8% of the total.

Adults

The newly emerged female is of a light shade of red, but within a few hours the color begins to turn darker and by the second day is dark red or chocolate brown. The body is full and decidedly rounded on the dorsum; the eyes are red.

The male is light yellow in color, but after feeding is usually greenish. The body is much smaller and more slender than that of the female and tapers sharply toward the caudal extremity. The eyes are red and prominent.

The adult male upon emerging is very active and soon seeks a quiescent female deutonymph. As a rule the male will remain in attendance until the female emerges, and it is not unusual for the male to wait in this manner from one to two days. Mating takes place immediately the female emerges, in fact the amorous male frequently assists the female to free herself from the moulting skin. A battle royal between the males is a common occurrence. This always proceeds until one is defeated and frequently more or less disabled. On one occasion the defeated male showed but little life for some time and after more than an hour could only feebly crawl.

The males are polygamous. A male in one instance mated with three females all within an hour. It was observed, however, that the last copulation period lasted only about two minutes, whereas the usual time was eight to twelve minutes. On another occasion a male mated twice during the afternoon and again with a virgin female the next morning. None of the females accepted more than one male.

After mating, the females feed on the leaves, extracting the leaf juices and depleting the foliage of chlorophyll or green coloring matter. The older leaves at the base of the current year's growth are preferred by the mites, and these as a rule are the first to lose the deep green and become pale and dull in color. As many as 140 adult females have been counted on these older leaves with only 5 to 20 at or near the terminal growth. Late in autumn, on sunny but otherwise cool days, the females congregate on the upper leaf surface where they remain for hours exposed to the sun's rays.

The adults were frequently observed crawling from a leaf by the way of the petiole to adjoining foliage. It appears, however, that this is, for the most part, the limit of movement.

The females in their movement over the limbs, in the process of laying winter eggs, spin some very fine silk. This webbing was only observed where the females were particularly numerous and it then extended along the limbs, over and about the massed eggs. Numerous eggs were found attached to this webbing. The silk was apparently easily removed from the trees by winds or storms for it disappeared soon after oviposition.

The length of life of the females and the number of eggs deposited vary with the different generations. For instance in 1931 the females of the second generation lived an average of 13.24 days with a maximum of 17 days. This was in contrast with an average of 24.25 days and maximum of 36 days for

the females of the fifth generation. In 1930, the greatest number of eggs were deposited by the females of the second generation, and the smallest number by those of the fifth generation, the averages being 34.92 and 9.74 eggs respectively. Ordinarily, the pre-oviposition period was one to three days but this also varied somewhat with the different generations. The usual rate of egg deposition was one to as many as four eggs each day during a period of eight to ten days. There were days, however, when no eggs were deposited, this being observed more particularly with the fifth generation. Oviposition was observed at a temperature of 44° F. Virgin females oviposited and the eggs hatched but the resulting mites were all males. Whether these mites were capable of fertilizing females was not determined. Parthenogenesis has been recorded by Newcomer and Yothers (11), Banks (1), and McGregor and Newcomer (10).

The males are much more active than the females and move rapidly over the leaves. There was only a short period at the height of emergence when males were numerous, for after mating they disappeared quite rapidly, under natural conditions. In the rearing cages the males usually disappeared within from one to three days after mating. No accurate records, therefore, were obtained regarding the length of life of the adult males, but all evidence indicates a much shorter period than for the females.

Immature Forms

The immature or developing mite passes through three distinct stages or instars, known as larva, protonymph and deutonymph.

The larvae are readily distinguished from the other instars as they have only six legs. After the larvae moult there appears the full number of legs,–eight. Each instar is divided into two well defined periods, first an active feeding one, followed by a period of quiescence. The mite is dormant during the quiescent period. The legs are rigid, partly drawn under the body, whitish in color and rather transparent. Most of these quiescent forms are found on the under surface of the leaf. They also occur to some extent on the upper surface, and occasionally on the nearby fruit spurs. A few hours previous to moulting, the quiescent forms usually become quite silvery in appearance owing apparently to the outer skin becoming separated from the body within. When moulting, the skin splits across the back in order to allow the mite to escape. The cast skins are eventually blown away or washed off by rains, but they frequently accumulate and are quite conspicuous, particularly on the under surfaces of leaves.

The sexes are not easily distinguished until the last instar, when they can be separated quite readily. The deutonymph males are smaller and more slender than the females, and the abdomen of the male is more pointed posteriorly.

Upon hatching, the young larvae crawl to the limbs and soon start feeding on the tender leaves. They prefer to feed upon the under surface although in the orchard many are to be found on the upper surface. At the commence-

ment of the rearing work many breeding cells were clipped to the upper leaf surface, but in every instance the mites became restless and wandered about the cell and finally escaped or became entangled in the sticky material. These cells were, therefore, discarded and all mites reared upon the under surface of the leaf. There was no extensive movement of the immature forms over the trees; in fact, there does not appear to be even migration from leaf to leaf unless there is an overcrowded condition. When this occurs there is a movement of the mites starting at or near the base of the current year's growth and proceeding toward the terminal. As they slowly proceed in this manner the leaves left behind show the characteristic mite injury of depleted chlorophyll and pale foliage.

The length of the larval-nymphal period varies somewhat with the different generations, that of the first and fifth being longer than the others. This has been largely attributed to the cooler weather prevailing in the spring and autumn. The length of the quiescent periods in the different instars is only slightly less than the active feeding period. This is shown in Table IV.

Hatching of the Winter Eggs

Daily records were taken of dates of hatching of the winter eggs of the European red mite for the seasons of 1930, 1931 and 1932 at Annapolis Royal. Hatching began in 1930 and 1931 on May 12, and in 1932 on May 15. This period of mite emergence lasted approximately two weeks. The weather has

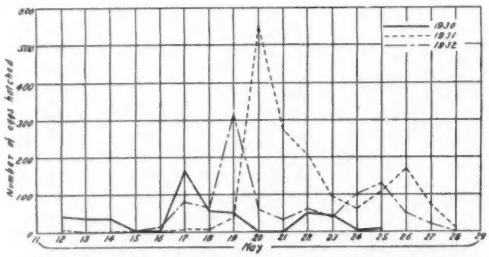

FIG. 2. *Diagram showing rate of hatching of winter eggs of the European red mite during the seasons of 1930, 1931 and 1932 at Annapolis Royal, N.S.*

a marked effect upon emergence, for the hatching of the winter eggs in 1930 was practically suspended during cold, wet periods on May 15 and 16 and again on May 20 and 21. Similar results were recorded during 1931 and 1932. During these cold periods not only is hatching delayed but the young mites, already emerged, cling tenaciously to the foliage, in a more or less semi-dormant condition with little apparent feeding or development. This results in the later emerging mites catching up, so to speak, with those emerging earlier and the disparity in dates of emergence of adults is not as great as might be expected.

Mortality of Overwintering Eggs

Data on the mortality of winter eggs were obtained in an orchard at Berwick in 1930 and in the insectary in 1930 and 1931. The insectary records were secured from twigs placed in vials containing water, some little time prior to egg hatching. The frequent handling of the twigs, in making daily counts of the emerging mites at the insectary, caused some injury to the eggs owing to their fragile nature. This has probably resulted in a somewhat higher mortality than ordinarily would occur. On the other hand, the direct orchard counts were not entirely satisfactory, as little control over natural factors seemed possible. The results are presented in Table III, but due allowance must be made for experimental error.

TABLE III

MORTALITY OF WINTER EGGS OF THE EUROPEAN RED MITE, 1930 AND 1931

Year	Records at	Total no. eggs on twigs	Number eggs hatched	No. eggs failed to hatch	Mortality, %
1930	Insectary	1832	936	896	41.10
1930	Orchard	1740	1448	292	16.72
1931	Insectary	2286	1608	678	29.60

Immature Forms

Daily observations were made of the mites in the rearing cells and all changes in development recorded from the time of hatching of the winter eggs until the adults emerged. It has not been convenient to include in this paper the individual rearing records of the immature forms, owing to the space involved. A summary is, however, presented in Table IV, showing the average duration of the various periods in the development of the immature forms in 1930 and 1931.

TABLE IV

COMPARISON OF FEEDING AND QUIESCENT PERIODS OF IMMATURE STAGES OF THE EUROPEAN RED MITE, ANNAPOLIS ROYAL, N.S.

Year	Generation	Larva		Protonymph		Deutonymph		Total average length immature period	
		Average feeding period, Days	Average quiescent period, Days	Average feeding period, Days	Average quiescent period, Days	Average feeding period, Days	Average quiescent period, Days	Feeding, Days	Quiescent, Days
1930	First	3.50	4.15	2.91	3.03	2.24	1.53	8.65	8.71
	Second	1.89	1.45	1.57	1.41	1.36	1.51	4.82	4.37
	Third	1.94	1.45	1.74	1.41	1.96	1.63	5.64	4.49
	Fourth	2.26	1.90	1.76	1.71	2.00	1.88	6.02	5.49
	Fifth	2.50	2.31	2.75	2.53	2.61	2.57	7.86	7.41
1931	First	2.71	2.66	1.86	2.10	1.57	2.37	6.14	7.13
	Second	2.21	1.93	2.20	1.71	2.19	1.57	6.60	5.21
	Third	2.31	1.70	1.98	1.77	1.83	1.45	6.12	4.92
	Fourth	2.40	1.47	2.01	1.84	2.37	1.88	6.78	5.19
	Fifth	3.28	2.43	2.41	2.26	3.31	3.31	9.00	8.00

Oviposition and Hatching of the Summer Eggs

In Fig. 3 are shown the periods at which oviposition and hatching of the different generations of the European red mite occurred in 1930 and 1931. A comparison of the laying and hatching dates shows uniformity for those two years. This was probably due, in a large measure, to the similarity of the seasons 1930 and 1931; both opened earlier than normal, and at about the same dates. The autumn in both years continued much milder than the average season, with no severe frosts at Annapolis Royal, until October 20. The average mean temperature was, of course, several degrees lower at the time of the fifth or last generation than earlier in the season; and it will be noted that the laying and hatching periods are more drawn out than those of the other generations.

Incubation of the Summer Eggs

The length of the incubation period varies somewhat with the different generations. This fluctuation is apparently largely due to the temperature factor, the midsummer generation showing the shortest period of incubation, with the last or autumn generation being several days longer. In Fig. 4 is shown the length of the incubation period of the summer eggs for all generations in 1930 and 1931.

In order to bring them together in condensed form, there is presented in Tables V and VI a summary of the more important phases in the life history of the European red mite, for the years 1930 and 1931. In the first generation, the incubation period is of necessity not included in the seasonal averages as the young mites of this generation emerge from the winter eggs. The average length of the complete life cycle of the first generation is therefore reduced accordingly. The complete life cycle is several days longer in the first and fifth generations than in those intervening. The oviposition period is also of longer duration early and late in the season. The number of eggs deposited is greatest in the mid-season generations.

TABLE V

SUMMARY OF THE PHASES IN THE LIFE OF THE EUROPEAN RED MITE, ANNAPOLIS ROYAL, N.S., 1930

Generation	Average length of stages		Pre-oviposition period,	Average length of complete life cycle,	Average length of oviposition period,	Average number of eggs laid by each female	Average length of life of female,
	Incubation, Days	Immature forms, Days	Days	Days	Days		Days
First		17.62	1.73	19.35	7.66	22.16	11.75
Second	9.21	9.20	2.18	20.59	10.66	34.92	14.53
Third	9.53	10.10	2.34	21.97	9.57	29.22	12.61
Fourth	9.81	11.45	3.22	24.48	10.62	17.65	16.20
Fifth	11.49	15.07	3.79	30.35	10.71	9.74	16.74
Average	10.01	12.69	2.65	23.35	9.84	22.74	14.36

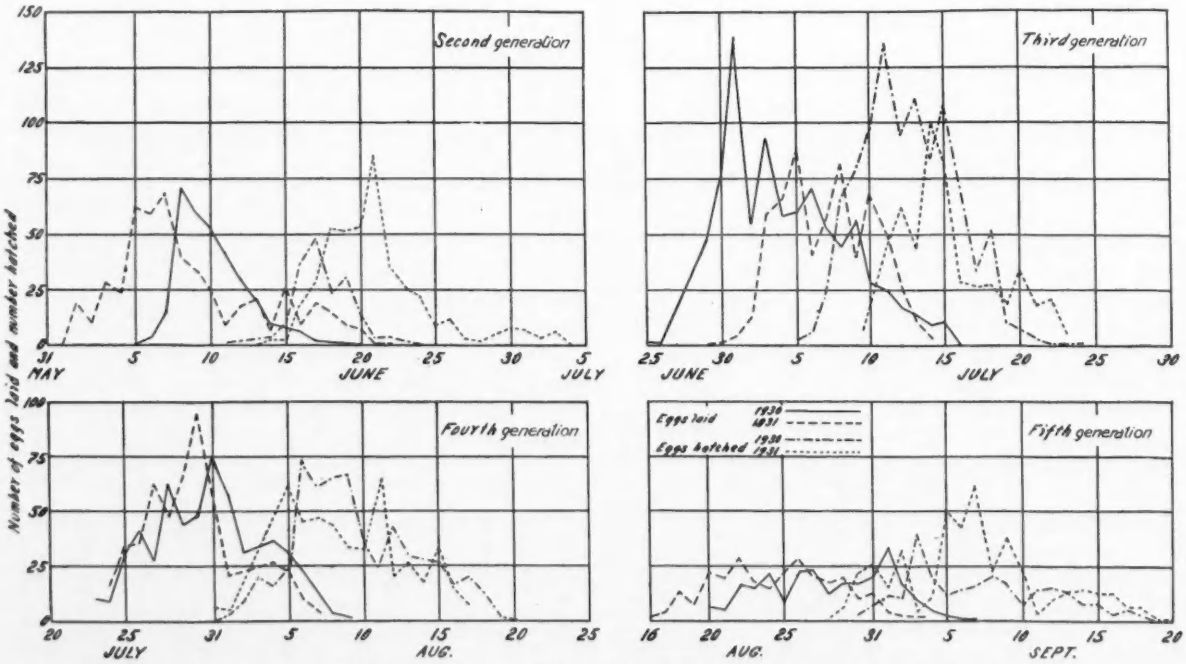

FIG. 3. *Diagram showing rate of egg deposition and rate of hatching of generations of the European red mite during the seasons of 1930 and 1931, at Annapolis Royal, Nova Scotia.*

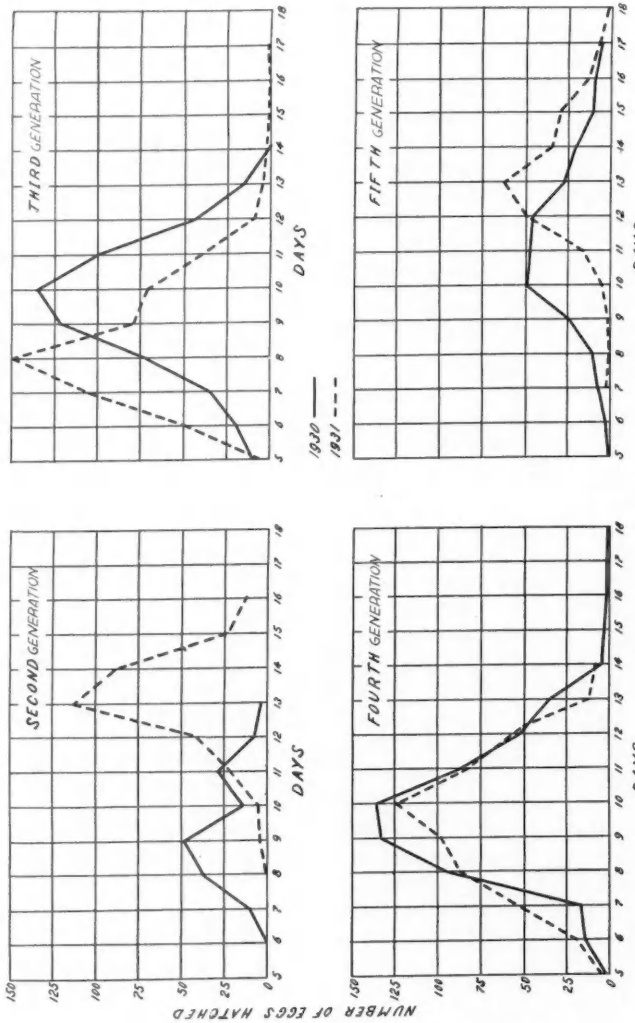

FIG. 4. *Diagram showing length of incubation period of generations of the European red mite for the seasons of 1930 and 1931, Annapolis Royal, Nova Scotia.*

TABLE VI

SUMMARY OF THE PHASES IN THE LIFE OF THE EUROPEAN RED MITE, ANNAPOLIS ROYAL, N.S., 1931

Generation	Average length of stages		Pre-oviposition period,	Average length of complete life cycle,	Average length of oviposition period,	Average number of eggs laid by each female	Average length of life of female,
	Incubation,	Immature forms,					
	Days	Days	Days	Days	Days		Days
First		13.20	2.75	15.95	9.88	24.00	13.20
Second	13.14	11.77	2.93	27.74	8.90	27.20	13.24
Third	8.40	11.10	2.00	21.50	9.00	28.58	13.61
Fourth	9.49	11.97	4.22	25.68	9.55	19.63	16.32
Fifth	13.03	16.90	7.16	37.09	13.37	6.63	24.25
Average	11.01	12.98	3.81	25.59	10.14	21.20	16.12

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